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VOLUME 5 NUMBER 1

GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS BEARING ON HEREDITY AND VARIATION

JANUARY, 1920

Princeton University Press
Princeton, New Jersey

GENETICS

A Periodical Record of Investigations Bearing on
Heredity and Variation

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Dr. Kölreuter.

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CORRIGENDA

Page 18, line 10, for "wheras" read "whereas", and for "T'_3" read "T'_2".
Page 30, line 10, for "germ splasm" read "germ plasm".
Page 38, line 2 from bottom, for "lines 1 and 2" read "line 1".
Page 44, line 9 from bottom, "41" should be light-face like the denominator.
Page 60, line 7 of table of contents, for "Selection in line 500. . . .64" read "Selection line 500 . . . . 66".
Page 60, line 8 of table of contents, for "67" read "69".
Page 60, line 9 of table of contents, for "68" read "70".
Page 60, line 10 of table of contents, for "70" read "72".
Page 60, line 11 of table of contents, for "71" read "73".
Page 60, line 12 of table of contents, for "73" read "75".
Page 60, line 13 of table of contents, for "74" read "76".
Page 60, lines 14 and 15 of table of contents for "75" read "77".
Page 180, line 15 of text, "10.648" should be raised to the position of an exponent of the quantity in parenthesis.
Page 190, line 20, insert semi-colon after the word "present".
Page 195, figure 4 is inverted.
Page 195, line 2 below the figure, for "*Pholox*" read "*Phlox*".
Page 211, line 9, for "magneta" read "magenta".
Page 215, last 3 lines of first column, for "$(AB)_{251\cdot 7}$" read "$(AB)_{250\cdot 20}$" for "$(AB)_{250\cdot 20}$" read $(BA)_{269\cdot 11}$"; and for "$(AB)_{269\cdot 11}$" read $(AB)_{251\cdot 7}$".
Page 217, line 9, for "anthocayanin" read "anthocyanin".
Page 222, line 6 from bottom, for "$(BA)_{269\cdot 1}$" read" $(BA)_{269\cdot 12}$".
Page 223, line 4 below table, for "5" read "25".
Page 228, line 11, insert comma after "D_e".
Page 237, line 7, for ".8 of" read "about".
Page 239, lines 1 and 2 below table, at right only, for "III a" read "III b".
Page 246, line 6 of paragraph 5b, for "*P*" read "*P* or *D*"
Page 247, line 6 of Literature cited, for "131 pp." read "154 pp."
Page 340, graph of chromosome, at extreme right only, for "*p*" read "*p'*".
Page 340, line 1 below graph, for "th" read "the".
Page 353, heading of table 5, third column from left, insert "and" before "Type A."

CORRIGENDA (CONTINUED)

Page 355, table, 6, between columns 1 and 2, insert a column as follows:

Agouti b.-e.-white *A W*
64 *76.5*

Page 355, column 3 of table 6, for "41" read "76," and for "*38.25*" read "*76.5*".
Page 355, column 4 of table 6, for "76" read "64".
Page 458, plate 2, a dotted line should connect P_5, No. 15 with P_4, No. 8.
Page 462, line 3, for "minature" read "miniature".
Page 466, line 6 from bottom, for "page 000" read "page 478".
Page 467, line 14 from bottom, for "minature" read "miniature".
Page 476, line 13 from bottom, for "othe frraction" read "other fraction".
Page 481, line 4 from bottom, for "minature" read "miniature".
Page 486, line 3 of paragraph 8, for "minature" read "miniature".
Page 558, line 7 from bottom, for "F" read "F_2".
Page 575, line 4 below table, for "20A–8–5–28" read "20A–8–5–22".

JOSEPH GOTTLIEB KÖLREUTER

(FRONTISPIECE)

This first great geneticist was born at Sulz-am-Neckar, Württemberg, Germany, April 27, 1733, and died at Karlsruhe, Baden, November 12, 1806. In 1748 he entered the UNIVERSITY OF TÜBINGEN, from which he received the degree of Doctor of Medicine in 1755, having spent one of the intervening years at the UNIVERSITY OF STRASSBURG. In 1756 he went to St. Petersburg where he had charge of the extensive collections of the IMPERIAL ACADEMY OF SCIENCE, and while there published some 20 notes and brief memoirs, chiefly on zoölogical subjects. In 1761 he returned to Germany as Professor of Natural History, first at Calw, and then in 1764 at Karlsruhe, where he was appointed Director of the Gardens of the Margrave KARL FRIEDRICH, a position which was rendered unhappy and finally untenable by the jealousy of the gardeners whom he superseded in authority. Although he ceased to be Director of the Gardens in 1769, he continued under the patronage of the Margrave throughout the rest of his life.

While at St. Petersburg, KÖLREUTER achieved success in the production of plant hybrids. His cross between *Nicotiana rustica* ♀ and *Nicotiana paniculata* ♂ was made in 1760, and 78 of the young hybrids were grown the same year. Twenty of these were brought to flower in-doors in March, 1761. With the exception of a single cross made by LINNAEUS (1757) between two species of Tragopogon, these were the first plant hybrids ever produced as the result of a fundamental genetical experiment, though at least one authentic hybrid was known to horticulture as early as 1719 (FAIRCHILD's pink).

These hybrids were intermediate between their parents in all the more striking differential characters, and the pollen was scanty or wanting. Similar phenomena were found to be generally the characteristics of species hybrids, and while there are some notable exceptions, the occurrence of pollen sterility in hybrids has been recognized as an important index of the specific distinctness of the parents, and this test is known as "Kölreuter's method."

KÖLREUTER's second Nicotiana cross (*Nicotiana transylvanica* × *N. glutinosa*), stands as the classic example of the excessive vigor often seen in hybrids, the phenomenon now known as "heterosis."

While most of KÖLREUTER's hybrids were identical in reciprocal crosses, he secured notably dissimilar reciprocals in Digitalis; and in Verbascum he met and recognized the phenomenon of self-sterility. In one of his later papers he also distinguished between "constant" and "inconstant" hybrids, but evidently did not appreciate the importance of this distinction. J. BEHRENS, who has prepared the only comprehensive biography of KÖLREUTER,* credits him with 283 successful hybridization experiments, of which the greater number involved Mirabilis (82), Dianthus (54), Nicotiana (43), Verbascum (36), and the Malvaceae (20).

Besides his strictly genetical experiments, KÖLREUTER made careful studies of pollen, of whose form and structure he gave descriptions, but owing to the inadequacy of the microscopes then available, he did not discover pollen-tubes, and so gained an erroneous conception of the nature of the fertilization process, which he thought to be a mingling of a female fluid secreted by the stigmas and a male fluid expressed from the pollen grains.

---

* BEHRENS, J., Joseph Gottlieb Koelreuter. Ein Karlsruher Botaniker des 18. Jahrhundert. Verhandl. d. naturwiss. Vereins in Karlsruhe 11: 268-320. *1 portrait.* 1894.

He also made numerous observations and experiments on nectar, and recognized and stated repeatedly and with emphasis the relation of insects to plant pollination. In this he anticipated by nearly 30 years the brilliant work of Sprengel (1793). Sprengel's "Discovered secret of nature" was no secret to Kölreuter nor to those who had read the published accounts of his experiments.

Only four of Kölreuter's papers are generally known, namely, his "Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen," and three "Fortsetzungen," which were published, respectively, in 1761, 1763, 1764 and 1766. These four papers were republished in 1893 in Ostwald's Klassiker. A note in the latter intimates that these were the only papers published by Kölreuter on his hybridization experiments, but this is erroneous, as he wrote between 1772 and the time of his death, 22 botanical and several zoölogical papers, mostly relating to hybridizations in various groups of plants. Nearly all of these were published in the Acta Academiae Scientiarum Imperialis Petropolitanae, St. Petersburg.

Kölreuter's strict adherence to the experimental method, his carefully planned and executed experiments, his direct and lucid statements of results, and the clear logic of his inductions and deductions, were strictly modern and in striking contrast to the writings of most of his contemporaries.

The portrait here reproduced is the only one of Kölreuter generally known, and it has been repeatedly engraved. We have been unable to secure information as to the present location of the oil painting which forms the basis of all these various engravings, but in 1894, when it was first reproduced, it was in the possession of W. Kölreuter, a grandson of J. G. Kölreuter, Hornberg, Baden, and it is probably still in the family. A second oil painting which is practically a mirror image of this one, is included among the relics of C. F. Gaertner which were deposited by Gaertner's daughter in the Botanischen Institut of the University of Tübingen. It is not known whether this second portrait is an original or whether it was copied from the one already mentioned, but Professor E. Lehmann to whom we are indebted for photographic copy of this potrait is inclined to believe that it is the original and the only *original* portrait of Kölreuter. He suggests that the facing of the engraved portrait to the right instead of the left was a result of the process of engraving. If this explanation is correct, certain other minor differences must be explained in the same manner.

The engraving used as the basis of our frontispiece was retouched and changed from an oval to an oblong setting by J. Marion Shull, of the U. S. Department of Agriculture. The fund necessary for its reproduction was generously provided by Professor Oakes Ames, of Harvard University, well known for his numerous papers on the Orchidaceae.

THE GENETIC BASIS OF TRUNCATE WING,—AN INCONSTANT AND MODIFIABLE CHARACTER IN DROSOPHILA

EDGAR ALTENBURG
The Rice Institute, Houston, Texas
and
HERMANN J. MULLER
Columbia University, New York City

[Received August 4, 1919]

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THE INITIAL SELECTION EXPERIMENTS AND CROSSES

One of the first mutants which MORGAN found in Drosophila was a fly with truncated wings. It appeared in 1910, in a stock having beaded wings, and the inheritance of truncate, like that of beaded, seemed from the first to be irreconcilable with Mendelian principles, or indeed with any theory of fixed and segregating factors.

The truncate and the beaded cases thus stood as a constant challenge to those workers who hoped for a completely rational explanation of in-

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heritance in Drosophila. Beaded has been dealt with in another paper; the truncate case will be considered here.[1]

The original truncate male, when crossed by its normal sisters, gave, even in F_1, a motley collection of offspring, the great majority being normal, but truncates of various grades also appearing. In F_2 the results were varied, and some of the F_1 matings of truncate by truncate gave nearly half the F_2 flies having some degree of truncation. It was to be expected that in the next generation pure stocks could be established; nevertheless crosses of the F_2 failed to produce any higher proportion of truncate than before, and for a few generations it seemed as though selection might be ineffectual. Broods yielding a higher percentage were found after several months, however, and by carrying on the selection work with these lines for about a year, MORGAN finally was able to obtain a stock in which sometimes as much as 90 percent of the flies showed the mutant character. The intensity of the character had increased markedly at the same time, but was accompanied by a greatly diminished fertility of the flies.

At this point one of the authors took up the work, and in an effort to obtain pure stock the process of selection was continued for nearly three years longer. It seemed, however, as though the climax had already been reached, for it was found impossible entirely to eliminate the normal individuals from the stock, or to obtain races that consistently yielded more than about 90 percent of truncates of all grades. It would not be justifiable to publish the protocols of these experiments in detail as this sort of breeding work is necessarily of an uncritical nature, not adapted to give definite factorial solutions to genetic problems. Table 1, however, is given as a sample of this series of experiments; it shows the effects simply of 13 successive generations of the selection, carried on during the latter part of the period. It will be seen that the average proportion of truncates (all grades) shows no significant change, being 84 percent for the first four generations, 90 percent for the intermediate five, and 88 percent for the last four.

These results suggest that this final stock might really be pure genetically, and homozygous for truncate, and that the differences between the individuals were due only to external conditions which accentuated or inhibited the development of the somatic character. In that case the normals and the truncates of various grades should all give identical results

---

[1] A preliminary account of the truncate case was given by the authors in the book of MORGAN, STURTEVANT, MULLER, and BRIDGES (1915, pp. 191-194). Most of the crosses there reported were made in 1913.

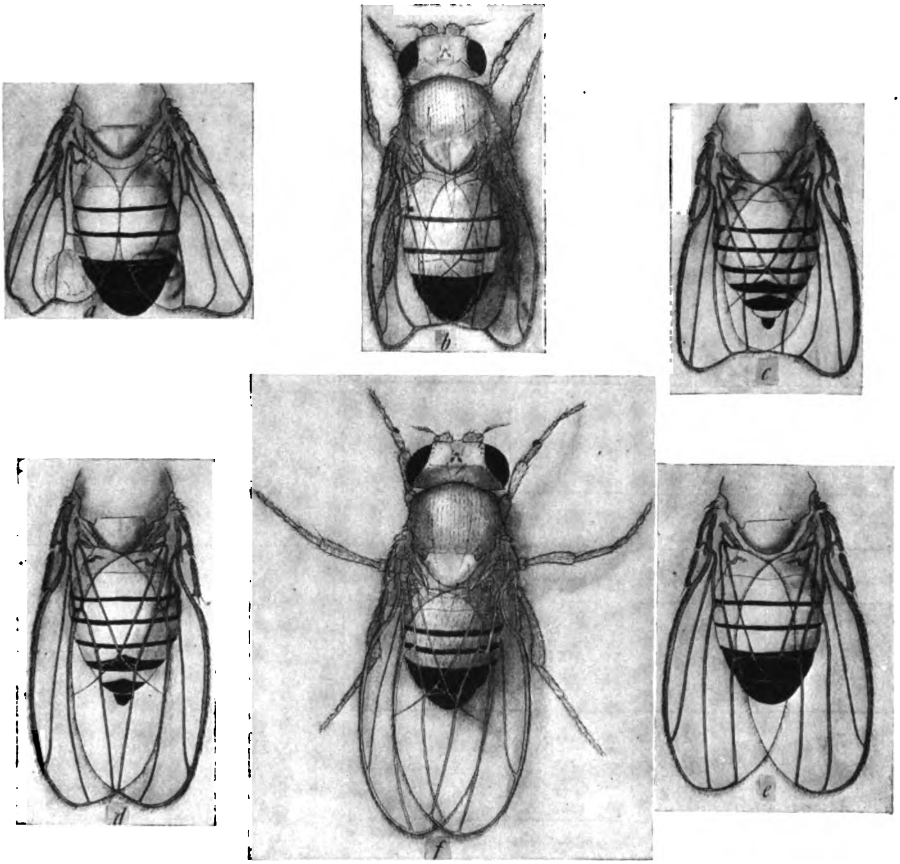

FIGURE 1.—*Types of truncation.*

a. Very short truncate male (the blistering is a frequent characteristic of this grade).
b. Typical short truncate male of the selected stock.
c. Long truncate female—the type of "truncate" usual in outcrosses.
d. Intermediate-winged female.
e. "Slight intermediate" male.
f. Normal-winged male from wild-type stock.

when bred. Table 2, however, demonstrates the wide genetic difference between the normals and the truncates thrown by this stock, for whereas the truncates on the average had given 91 percent of truncates in this experiment, the normals yielded only 5 percent. Even the selected stock therefore is undergoing a continual genetic variation, with which the variation in the character is correlated, and so selection back and forth within this stock still has immediate and marked effect, within the 90 percent limit.

TABLE I

"Main line" of truncates selected for 13 generations for homozygosis.

Number of generation	Truncates (all grades)	Normals	Average grade of truncation according to generation	Average grade according to groups of generations	Average percentage of truncates
1	*13	0	3.0	2.52	84
2	5	0	2.3		
	*19	4			
	16	8			
3	*11	0	2.7		
	15	1			
	12	3			
4	*36	8	2.5		
5	*18	2	2.7	2.70	90
6	23	3	2.5		
	8	2			
	*28	8			
7	*98	6	2.8		
	9	2			
	53	6			
8	*13	2	2.6		
9	*8	0	3.0		
10	30	3	2.6	2.64	88
	*19	4			
11	10	1	2.5		
	*29	7			
12	*79	10	2.7		
13	28	1	2.9		

* The asterisks denote those families from which the parents of the families recorded in the next generation were taken. It will be seen that, in some cases, as in generation 6, the family thus marked out is not the one that shows the highest percentage of truncates. Such a curcumstance is due to the infertility of the "best-truncate" families; attempts were made in each generation to breed from the "best" families, as well as from the others, but the "best" lines frequently died out after one or several genera-

tions, the race then being continued from the families of those poorer lines which had been carried on simultaneously as a safeguard. The above table shows none of the unsuccessful side lines which were in this manner always being carried on, both from better and from poorer stocks, but it gives a record only of those families that happened to lie in the line that was carried through continuously.

As a typical example of an unsuccessful side line, it may be stated that in generation 6, referred to above, three cultures were made of the truncate flies in the "best family" (that which consisted of 23 truncates, 3 normals). The "best" of these three cultures gave, in generation 7, 78 truncates and 1 normal; two families in turn were started from this culture; of these, one produced nothing at all, and the other yielded only three infertile flies in generation 8. The "main line" thus reverted to the descendants of the third bottle of generation 6, which has therefore been marked with the asterisk.

In determining the average grade of truncation of the above counts, the class of truncate is assigned an average grade of 3 and the class of normals a grade of 0, each grade is then multiplied by the number of flies in the class having that grade, and the sum of these products is divided by the total number of flies.

TABLE 2

Genetic behavior of normals thrown by truncates of the selected stock.

Number of generation	Truncates	Normals
1	38	3
2	21	1
3	17	1
4	62	9
5	0	27
6	1	19
7	5	67

Lines 1 to 4 show the counts given by the truncate stock for the four generations preceding that in which normals were chosen; only those families are here recorded which are in direct line with the one (4) from which the normals were derived. Lines 5 to 7 show the ratios thrown by the normals during three successive generations in which they were selected as parents. Individual matings (separate pairs of flies) were not used in this experiment.

The results of crossing truncate to wild-type flies were extraordinarily indefinite and inconclusive. A few examples of the numerous crosses of this sort which were carried out are given in table 3. Truncate behaves nearly as a recessive in F_1 (see table 3(A)), although a small percentage of flies appeared with the character more or less perfectly developed. In F_2 a good 3 : 1 proportion was never secured, but, as may be seen from table 3(B), ratios occurred varying from 168 normals : 1 truncate, to less than 2 normals : 1 truncate, the "truncates" being of various grades. In other crosses of the same type F_2 families have also occurred in which no truncates were produced at all. When an attempt was made to recover truncate stock by breeding together the "extracted" truncates

TABLE 3

Crosses of truncate by wild stock.

(A.) F_1 (from wild female by truncate male)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
1	Wild ♀, T ♂ from selected stock	5	1	4	2	162	182	3.36
2	Wild ♀, T ♂ from selected stock	5	0	1	3	129	127	3.4
3	Wild ♀, T ♂ from selected stock	0	1	1	0	89	83	1.15
4	Wild ♀, T ♂ from selected stock	4	1	1	3	214 (♀&♂)		4.03
5	Wild ♀, T ♂ from early unselected stock	0	0	0	0	672	676	0.0

(B.) F_2 (from $F_1 \times F_1$)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
6	T ♀, normal ♂ from 3	22	9	3	4	103	68	18.2
7	" " " " " 4	15	11	7	1	32	21	39.2
8	I " " " " 3	28	15	20	14	173	120	20.8
9	N ♀, N ♂ both from 3	17	6	3	3	184	174	7.5
10	N ♀, N ♂ " " 5	0	2	0	0	208	128	.6
11	" " " " " " 5	12	20	3	5	168	125	12.0

(C.) F_3 (from $F_2 \times F_2$)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
12	T ♀, T ♂ both from 6	85	49	14	13	54	42	62.6
13	" " " " " " 8	101	43	13	10	77	111	47.04
14	N ♀, N ♂ " " 8	37	22	23	22	158	172	23.0

(D.) F_4 (from $F_3 \times F_3$)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
15	T ♀, T ♂ both from 12	132 (♀&♂)		11 (♀&♂)		44 (♀&♂)		76.5
16	" " " " " " "	79	58	43	42	1	8	63.0
17	" " " " " " "	32	17	1	0	112 (♀&♂)		30.9
18	N ♀, N ♂ " " "	35	31	4	5	50	73	37.8
19	" " " " " " "	0	0	0	0	464 (♀&♂)		0.0

of the F_2 families, it was found (table 3(C)) that truncate had been considerably "weakened," as only about 50 to 60 percent of truncates were now produced. Further selection, however, brought the extracted stock in later generations nearer to the value of the original selected stock. (An indication of this may be seen in table 3(D).) It was found that the percentage of truncates in F_2 and later generations of the cross tended to be higher if the P_1 truncate fly had been a female, than if the reciprocal P_1 cross had been made, and yet fair numbers of truncates of each sex were produced in both cases. All these results could be "explained" as due to varying "potencies," but this did not put the matter on a factorial basis.

Besides being crossed to wild-type, truncate was also crossed to various mutant stocks, and counts were made of the F_1, and of the F_2 derived from interbred F_1. Here the same indefinite results were obtained, in respect to the truncate wing character, as in the crosses with wild, but in addition there was also observable an apparently indefinite sort of linkage between truncate and the other character, which appeared, curiously enough, regardless of the linkage group to which the other character belonged. As examples of such experiments, the results of some of the crosses with jaunty (group II), and with pink (group III), are given in tables 4 and 5 respectively. It will be seen that in the F_2 of the cross with pink, although some degree of linkage was clearly indicated, yet a surprisingly large proportion of pink truncates were produced in certain bottles,—more than could be accounted for by weakness of linkage and by the amount of dominance shown by truncate in F_1.

ANALYSIS OF THE EFFECTS OF THE DIFFERENT CHROMOSOMES IN TRUNCATE STOCK ON THE TRUNCATE CHARACTER

It was thus necessary to discover, first, whether truncate depended on definite factors, lying in the chromosomes, and, secondly, if this proved to be true, whether the continual genetic variation occurring in the stock was due to fluctuation in these factors, or to heterozygosis that for some reason was forced to persist in the stock. As it was evident that at any rate the results did not represent simple monohybrid ratios, a method was required whereby the different factors, or sets of factors, that might be concerned in producing truncate could be dissociated from each other and studied separately. This object was achieved by making use, in a special way, of the linkage relations which had been worked out in Drosophila.

Generation of count	Bottle numbe
F_1	1
F_1	2
F_1	3
F_2	4
F_2	5
F_2	6
F_2	7
F_2	8
Sum of F_2	

* The word
to truncation.

It may
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distribution of all the factors lying in the corresponding pair of chromosomes. This will tell nothing, however, about the distribution of the factors in the other three pairs of chromosomes, since the different pairs of chromosomes are distributed independently of one another to the offspring, and so there is opportunity for factors in different pairs of chromosomes, which may in previous generations have accompanied each other, to become dissociated from one another—i.e., transmitted to different offspring. Hence in order to know what was the distribution of all four pairs of chromosomes of a male parent among his offspring, it is necessary to have under observation the inheritance of four pairs of characters—one for each of the chromosome-pairs; each of these characters, then, will serve for the identification of an entire chromosome or group of factors in the offspring, and may be called an "identifying character." So, by this method, it is possible to determine the distribution, among the offspring, of the entire constellation of factors which a male parent contained. The offspring will contain different combinations of his factors (assuming he was heterozygous), but in each case it will be known just what combination is present. The somatic effects of these different known combinations of factors on the offspring may then be studied, and so it may be determined just what rôle each of the groups of factors plays in the production of any particular character that is under investigation. In this manner it was possible to ascertain what effect on the development of truncate wing each chromosome of the truncate fly produced.

It should be noted, however, that the simplicity of such an experiment is impaired when the character under investigation (e.g. truncate) is recessive, for then the factors for it must be introduced from the female parent as well as from the male, in order that the character may be observable in the offspring. The disadvantage of thus introducing the factors from the female parent is that in the female the factors for the character under investigation are liable to cross over from the identifying factors of the groups to which they belong, and it may therefore be impossible to tell, by means of one identifying factor in each chromosome, exactly what combination of factors for the character the offspring studied have received.[2] It would accordingly be desirable to have the character dominant, in order that in the investigation the factors for it may be introduced from the male parent alone. In the case of truncate, this mode of procedure was rendered possible by a special circumstance.

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[2] If, however, an identifying factor at each end of the chromosomes is used, it may be assumed with a fair degree of probability that the offspring receiving both identifying factors have also received those factors for the character studied which lay between them.

Ordinarily, truncate behaves most nearly like a recessive character, when truncates are crossed to wild-type flies, although a very small percentage of F_1 showing some degree of truncation are almost always produced. Examples of such crosses have been shown in table 3. Here the flies which have wings at all truncated have been classified, for greater accuracy, into two divisions—typical "truncates," and "intermediates," and it will be seen that the number in the two classes is approximately equal. The total proportion of truncates—including both grades—is 3.1 percent (in the crosses of truncate of the selected stock). When truncate was crossed to various other types of flies somewhat similar results were usually obtained, but when the cross was tried of truncate from the selected stock by flies with black body color, the outcome proved to be conspicuously different, as shown in table 6. Here it will be seen that there are distinctly more typical truncates than intermediates, and the proportion of truncates of all grades appearing in this cross is also very much higher, being in fact 24.9 percent (in the cross of truncate males by black females), as compared with the previous 3.1 percent (when truncate males were crossed to wild-type females). The truncation of the two series of F_1 flies may be compared also by obtaining a figure which may be called the "average truncation" of each group of individuals. The typical truncates may be assigned a

TABLE 6

Crosses of truncates by black.

(A) F_1 from black ♀♀ × truncate ♂♂

Bottle No.	Truncate		Intermediate		Normal		Percentage of T and I
	♀	♂	♀	♂	♀	♂	
1	35	0	11	13	90	116	22.3
2	57	0	25	18	113	162	26.6
3	55	1	22	12	121	149	25.0
Sum	147	1	58	43	324	427	24.9

Average truncation among ♀♀ 1.33, among ♂♂ .19, among all .79.

(B) F_1 from truncate ♀♀ by black ♂♂

Truncate		Intermediate		Normal		Percentage of T and I
14	11	7	0	25	12	46.4

Average truncation among ♀♀, 1.52; among ♂♂, 1.91; among all, 1.65.

grade or value of truncation of 4, in this calculation, the intermediates a value of 2, and the normals 0. Multiplying each of these values by the number of individuals having the value, adding, and then dividing by the total number of individuals, it is found that the average grade of truncation of the F_1 from black females by truncate males is .79, whereas the average grade of the F_1 in the cross of selected truncate with normal is .10. This relatively high grade of truncation for the F_1 flies has been obtained consistently, in numerous and varied crosses of truncate with flies of black body color. Moreover, although there is still a considerable proportion of normals present among the F_1 even in these crosses, it will be shown later that "high grade" truncate is really almost always dominant in individuals heterozygous for black. These results with black were in themselves noteworthy, for here there seemed to be a type of interaction of factors hitherto not described, wherein two non-allelomorphic factors, both ordinarily recessive, re-enforced each other so as to produce a visible effect in the individuals heterozygous for both, just as happens in crosses of multiple allelomorphs. It should be added that the black character, unlike the truncate character, was not affected by this interaction, and remained recessive. The facts were more important, however, in their bearing on the truncate case, for they disclosed a means whereby truncate might be used as a dominant character, in the desired analysis.

In order to simplify the account of the analysis, crosses are here reported in which only the three large groups of factors were followed,—the fourth chromosome, which was too small to be likely to contain factors for truncate, was followed, however, in other experiments, and proved not to be concerned in the case. The sexes were used as the pair of "identifying characters" for the first chromosome; black body color, *b,* versus *B,* the normal gray, for the second chromosome; and pink eye-color, *p,* versus *P,* the normal red, for the third chromosome. The procedure was as follows: A truncate female with normal body and eye colors was mated to a normal-winged male with black body and pink eyes. The F_1 males, the distribution of whose factors among the F_2 flies was to be followed, had the following chromosome and factor composition, indicating the chromosomes by numerals and representing those derived from the truncate parent with asterisks, and the homologous chromosomes, derived from the black pink parent, without asterisks:

$$\frac{\text{I}^*X}{\text{I}\ Y}\ \frac{\text{II}^*B}{\text{II}\ b}\ \frac{\text{III}^*P}{\text{III}\ p}$$

Inasmuch as these flies are heterozygous for black, it was to be expected that truncate would behave as a dominant, at least partially, and the count designated as "cross 1" in table 7 confirms this expectation, for 33.3 percent of the flies had somewhat truncated wings. It is not in this generation, however, but in the next, that the desired analytical results appear.

One of these F₁ flies (a male) was back-crossed to homozygous black pink females of the composition

$$\frac{\mathrm{I}X}{\mathrm{I}X}\ \frac{\mathrm{II}b}{\mathrm{II}b}\ \frac{\mathrm{III}p}{\mathrm{III}p}$$

in order to obtain offspring (F_2) having various known combinations of the chromosomes derived from truncate stock. As all these offspring (F_2) alike received the non-dominant combination I*X* II*b* III*p* from their homozygous mother, the circumstance of receiving I*X or I*Y*, II*B or II*b*, and III*P or III*p* from their heterozygous father determined whether their characters were female or male, gray or black, and red or pink, respectively. The visible characters accordingly revealed just what chromosomes of the truncate stock these flies had or had not received. Thus, the female gray red flies must have received from their father all of his truncate-derived chromosomes,—I*X, II*B, and III*P,—while the male black pink flies could not have received any of these chromosomes, but must have gotten the non-truncate combination I*Y* II*b* III*p* instead. Corresponding with these facts, we find that the former flies (gray red females) are very largely truncate in wing shape, whereas all the latter (black pink males) have normal wings. These are the two extreme combinations; all the others received from the heterozygous father certain chromosomes derived from the truncate stock and others derived from the normal-winged (black pink) stock; a study of the amount of truncation shown by each of the different combinations may then reveal the rôle played by each chromosome in the production of the truncate character.

The results of the cross are designated as "cross 3" in table 7. There are eight possible combinations of the three pairs of identifying characters, and it will be seen that all of these combinations, or classes, appeared with about equal frequency, just as expected. In the case of each class the flies were further subdivided into four different grades, according to their wing shape. These grades (normal, "slight" intermediate, intermediate, and "truncate") may be given average values of 0, 1, 2.5, and 4, respectively, to bring them into proper relationship with the three grades previously used. The fact most obvious on examining the records of the different classes, with regard to their amount of truncation, is that all of the black flies have normal wings; i.e., none of the flies in any of the last four combinations, which failed to receive II*, shows any indication of the truncate character; on the other hand, some at least of the flies in each of the first four, gray classes—the classes that did receive II*—had wings with some degree of truncation. This proves that there is a factor (or factors) for truncate in II*, i.e., in the second chromosome. This factor (factors?) in heterozygous condition is sufficient "by itself" to cause truncation, even if none of the other chromosomes in the fly are derived from truncate stock, but if this factor is not present, truncate cannot appear (at least, not as a dominant), even if one of the first and also one of the third chromosomes in the fly are derived from truncate stock. The factor for truncate in chromosome II is therefore the "chief" factor for truncate—being both necessary, and self-sufficient,[3] for the appearance of truncate (as a dominant). It may be designated as T_2'; its normal recessive allelomorph would then be t_2', and in our notation t_2' may ordinarily be understood to exist in any formula of the second chromosome in which T_2' is not represented.

T_2' is not the only factor for truncate whose existence is disclosed by this table. Comparison of the four classes which received T_2' (the gray classes) shows that truncate appeared in them with very different frequencies and intensities. It is evident that the red-eyed gray classes—those which contain III*P (the first two classes of the table)—contain more and better truncates than the corresponding pink-eyed gray classes—those having IIIp (the third and fourth classes of the table). The chromosome III* that came originally from truncate stock therefore contains a factor (or factors) for truncate also. This may be called T_3'; like T_2' it is dominant, inasmuch as it may produce an effect when in heterozygous condition. Unlike T_2' however, it may be called a "modifier" or "intensifier" rather than a chief factor, for it can produce an effect on the wing only in the gray classes, which already contain II*B (T_2').

It was then shown that even when an opportunity is given for it to exist in homozygous condition, T_3', without T_2', is unable to cause truncation. This was done by crossing the males and females from the black-bodied, red-eyed classes, to each other; the result is given in table 7, cross 5. The parent flies must all lack T_2' but are heterozygous for T_3'.

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[3] Within the limits of the experiment.

Among the offspring, therefore, one-fourth should be homozygous for T_3', yet none of the flies had truncate wings. Three had a very slight unevenness of margin that approached the condition seen in slight intermediates, but this did not seem to be transmitted to their offspring. T_3', then, has practically no effect on the wing except in the presence of T_2'. Moreover, T_3' is not really necessary for the production of truncation, since some of the pink flies of the main experiment—flies that carried T_2' but could not have received T_3'—are truncate, although of course truncation in them is not so frequent nor so intense as when T_3' is present. T_3' thus fulfills the definition of an intensifier.

In a manner similar to that in which T_3' was deduced, we may also infer the existence of at least one intensifier, T_1', in the first chromosome (I*X) derived from the truncate stock. For the tables show that the female classes—i.e., the flies with I*X—contain a much larger propor-

TABLE 7

Crosses to determine the effects of the different chromosomes of selected truncate stock.

(A) Initial crosses of truncate by black pink

Generation of count	Number of cross	Nature of parents ♀	♂	Gray red T	I	S	N	Gray pink T	I	S	N	Black red T	I	S	N	Black pink T	I	S	N
F₁	1	1 T from stock	Several bp from stock	3 4	8 7		18 26												
"	2	Several bp from stock	3 T from stock	13 1	10 12		39 56												
(B) Back-crosses to separate the effects of the chromosomes																			
F₂	3	Several bp from stock	1 T from 1	57 2	23 17	0 12	7 47	2 1	8 4	6 4	64 62	0 0	0 0	0 0	74 67	0 0	0 0	0 0	82 73
"	4	1 T from 1	Several bp from stock	2 0	1 1	1 1	6 2	1 0	3 5	1 1	4 4	0 0	2 1	0 0	10 4	0 0	0 0	0 2	9 2

TABLE 7 (continued)

(C) Crosses of derivatives of the separation cross

Generation of count	Number of cross	Nature of parents ♀	♂	Gray red				Gray pink				Black red				Black pink			
				T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
F$_2$	5	Several black red N from 3	Several black red N from 3									0 0	0 0	0 0	121 127	0 0	0 0	0 0	36 38
"	6	4 gray pink (2S&2N) from 3	1 black red N from 3	6 4	5 2	11 3	43 60	0 0	0 0	0 1	42 59	1 1	1 1	1 1	55 73	0 0	0 0	0 1	45 44
"	7	4 black red N from 3	1 gray pink S from 3	5 2	11 8	14 11	22 31	0 1	3 2	1 8	39 47	0 0	0 0	0 0	49 46	0 0	0 0	0 0	47 49
"	8	4 black pink N from 3	1 gray red S from 3	8 5	14 6	11 8	9 14	0 1	1 3	0 1	37 30	0 0	0 0	0 0	54 17	0 0	0 0	0 0	29 24
"	9	2 gray red I from 3	Several bp from stock	3 5	5 12	11 9	48 43	0 0	4 3	9 14	45 55	4 3	1 4	2 2	52 76	0 0	3 1	5 4	55 44

Females are recorded on the upper line, males on the lower, in the case of each cross. T = truncate, I = intermediate, S = "slight-intermediate," N = normal-winged; in section (A), both I and S have been grouped under I.

tion of truncates, and are more intensely truncated, than the corresponding male classes, provided of course that II**B* (T_2') is present.

It might be objected here, however, that in the case of the sex chromosome the difference between the truncation of the males and females may not be due to an intensifier present in I**X* and absent in I*X*, but that it may be due rather to sex difference, depending on the XX condition of the female and the XY condition of the male. This possibility was tested out by making reciprocal crosses of truncate to non-truncate flies. As will be seen from tables 4, 5, 6, and 7, the male offspring are distinctly more truncated (in comparison with their heterozygous sisters), when they have truncate mothers than when they have truncate fathers. In table 6, for instance, there was an average grade of truncation of .19 among the male offspring of truncate males, whereas the male offspring of the truncate female had a grade of 1.91—ten times as high. The daughters were not very different in the two crosses, being 1.33 in the first, and 1.52 in the second. The sons must therefore be able to receive a factor, or factors, for truncate from their mother, which they cannot receive from their father,—that is, there are one or more dominant factors for truncate that obey the scheme of sex-linked inheritance. There must consequently be one or more intensifiers, T_1', in chromosome I*; the symbol of the normal allelomorph, t_1', will ordinarily not be represented, but its presence may be taken for granted in all X chromosomes in which T_1' is not shown. T_1', like T_3', cannot cause truncation, even in homozygous condition, unless T_2' is present.

In the crosses in which the female parent was truncate the male offspring were nearly always somewhat more truncated than the female, because the males are haploid for sex-linked factors and therefore appear like homozygotes, whereas the females contain factors from both parents and show some effect of the normal allelomorph received from the father. T_1', in other words, is not a completely dominant factor.

Among flies which are homozygous in regard to the sex-linked factors, on the other hand, no matter whether they are pure for the intensifier, or for its normal allelomorph, the males are on the average less truncated than the females. This effect is especially evident where the normal allelomorph of T_1' is present and is clearly shown in tables 9 and 10 (to be discussed later in another connection), wherein the males and females are both lacking in T_1' throughout, and are like each other so far as all non-sex-linked factors are concerned. In these crosses the female parents were non-truncated and the male parents were heterozygous for T_2' and T_3', but lacked T_1' (having resulted from a cross of truncate male by non-truncate female). In table 9 (A) and (B), for example, it is easily observable that there is a great preponderance of females among the truncate and "good-intermediate" offspring; the intermediate class has about equal numbers of females and males, and the slight-intermediate and normal classes contain almost exclusively males. The grade of truncation among the 914 females in these two tables averages 3.2, whereas that among the 864 males averages 1.6. In table 10, the average grade of truncation in the 535 females is 3.1, that in the 517 males is 1.3.

We may say, then, that the character truncate is partially sex-limited, or rather, *sex-influenced,* its development being favored more in the female sex than in the male. Or, to put the matter in factorial terms, the sex-factor itself, or some factor normally lying in the sex chromosome, intensifies the development of truncate, to a degree depending upon whether this factor is present in one "dose" or two, just as is the case with sex itself. This fact has nothing to do with the existence of a mutant intensifier, T_1', in the same chromosome.

After truncate had thus been analyzed into its chromosomal components, it was demonstrated that it could also be resynthesized by breeding together individuals of non-truncated or imperfectly truncated classes (gray pink females, black red males, etc.),—classes which, according to the analysis, should contain "complementary" components. Thus, the gray pink females should contain T_1' and T_2', and the black red males just T_3'. By breeding them together, then, as good truncates should be obtained as were found among the gray reds, in fact, the count should be rather similar to that obtained from the heterozygous gray red females by black pink males, except that here there is a chance for crossing over between black and truncate. The count, designated as cross 6, table 7, is quite according to this expectation. It will be noted here that the male parent had been normal-winged, and the females were only slight intermediates or normal (not possessing T_3'); the knowledge of what results would be produced with respect to truncate was thus entirely derived from the identifying factors. Another, somewhat similar cross, was of black red (long) females by a gray pink (slight-intermediate) male. Here the male has T_1' and T_2', and the female T_3'. The count is shown on line 7 of table 7, and again shows the high-grade gray red truncates. Black pink females derived from the main experiment, and therefore containing T_1', were likewise crossed to one of their complementary brothers, a gray red male ($T_2'T_3'$). The count, on line 8 of the same table, shows typical truncates, as before.

These results are all in striking contrast to those of other crosses, in which the parental classes (derived from the same cross as those above) did not supply complementary elements; compare, for example, the case where black reds were crossed to each other (table 7, cross 5), with the crosses above. Crosses involving still other combinations of these classes will be presented later. In each case, the results conformed to the expectation based on the analytical findings.

Let us now return to the three crosses of "complementaries," for we shall find that closer scrutiny of them will give us some further information regarding the nature of the accessory factors for truncate. Crosses number six and eight, besides illustrating the reconstruction of truncate, provide an indication of whether the component designated as T_2' consists of a single factor, or a group of factors for truncate lying in the second chromosome. For, if T_2' is of multiple composition, a distinctly lower grade of truncate would be produced in cross six than in cross eight, since in the former cross T_2' is supplied to the offspring by their heterozygous mother, in which the process of crossing over would allow these supposed constituent factors of T_2' to become separated and scattered among different offspring, wheras in the latter cross T_3' comes from the father, and so its various factors would have to stay together (no crossing over), and re-enforce each other in the offspring, thus producing truncates of a higher grade than the average of the first cross. On the other hand, if T_2' is just a single factor, the truncates in the two crosses will of course be of the same grade. A test for T_2' alone is involved here, as the two crosses were identical (not reciprocal) so far as the chromosomes other than II were concerned, the cross six being $\frac{T_1'X}{X}\ \frac{T_2'B}{b}\ \frac{p}{p}$ female by $\frac{X}{Y}\ \frac{b}{b}\ \frac{T_3'P}{p}$ male, and the cross eight being $\frac{T_1'X}{X}\ \frac{b}{b}\ \frac{p}{p}$ female by $\frac{X}{Y}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ male. When the averages were calculated, it was found that the average truncation of the red truncated offspring (including all grades showing truncation) in cross six was 2.34 and that the average in cross eight was almost exactly the same, 2.33. Blacks and grays are both included in the average, since, on account of crossing over between black and T_2' in the third cross, the truncates are distributed among both black and gray classes. Taking all the truncated offspring,—black and gray, red and pink,—it is 2.26 in cross six and 2.34 in cross eight, a difference that is scarcely significant. These results then, indicate that T_2' is a single factor.

In a similar manner, comparison of crosses seven and eight give data regarding the composition of T_3', for in cross seven there is a chance of crossing over between the possible constituents of T_3', since T_3' is supplied by the female, whereas in cross eight T_3' comes from the male. while the crosses are identical so far as the other chromosomes are concerned. The grade of truncation of the red flies in cross seven was 1.97, and of all flies in that cross, 1.91. These rather low figures incomparison with values 2.33 and 2.34, that we have seen were obtained in cross eight, thus indicate that T_3' consists of more than one intensifying factor in the third chromosome. Other evidence for this was obtained in later crosses, when it was found possible to obtain, by crossing over, stocks of pink truncate which there was reason to believe contained an intensifier in the third chromosome, that was not as marked in its effect as the original T_3'.

From the various experiments reported above, it is evident that truncate is a character that is particularly susceptible to modification by various factors, such as T_1', T_2', T_3', and "sex" (in the sense previously explained). Even the symbols T_1', T_2', T_3', moreover, probably do not represent separately all the mutant factors for truncate that exist in truncate stock, since, as has just been shown, it is likely that T_3' at least is composite. And, in addition to such specific factors for truncate, and sex, it should be remembered that there are mutant factors concerned with the development of other characters, which influence truncate at the same time. Thus, we have seen that the factor for black body-color must be regarded as an intensifier of truncate, as truly as T_1' or T_3'. There is evidence that a number of other previously known factors behave somewhat similarly. For instance, when a truncate male was placed in a bottle containing at the same time both a virgin bar-eyed female and two virgin round-eyed females (all non-truncate), the bar-eyed offspring that hatched in this bottle (bar is a dominant) were distinctly more truncated than the round-eyed offspring. As the environments of the two sets of offspring must have been exactly the same, the factor for bar, or some factor in the bar stock, must be a dominant intensifier of truncate. This result is shown in table 8. The average truncation of the bar offspring was 1.44, that of the rounds was 0.[4]

TABLE 8

Cross of a single truncate male (selected stock) by various females in a single bottle. One female parent bar (dominant, sex-linked), one vermilion miniature (recessive, sex-linked), one wild type; all three normal-winged. Count of F_1.

Females								Males											
Normal-eyed				Bar-eyed				Normal-eyed				Bar-eyed				Vermilion miniature			
T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
0	0	0	73	24	3	2	28	0	0	0	65	15	4	2	45	0	0	0	20

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[4] It may be added that in this experiment one of the round-eyed females had been a vermilion-eyed miniature-winged fly (*v m*), and this female left offspring as well as the other female, as shown by the presence of some *v m* sons (*v* and *m* are sex-linked), yet these two mutant factors, unlike bar, failed to intensify the truncate character.

A somewhat similar experiment was performed with the dominant factor for star eyes (star is in chromosome II). Heterozygous star females were crossed to truncate males (of a rather low-grade pink truncate stock containing that part of T_3' which had crossed over with pink). The count of non-star offspring was 100 normal males, 80 normal females, no truncates of any grade; the count of stars was: males, 69 normal-winged, 27 slight intermediate, 12 intermediate, 1 truncate; females, 103 normals, 36 slight intermediate, 25 intermediate, 16 truncate.

TESTS OF THE CONSTANCY OF THE FACTORS FOR TRUNCATE

a. *Tests of sibs*

Although it had thus been established that truncate is certainly a complex case of multiple factors, nevertheless this alone did not seem to explain satisfactorily the final failure of the persistent effort that had been made to secure homozygous stock. For, in an animal in which all the factors are linked into only four groups, it should not have taken so very many generations to secure complete homozygosis, no matter how many factors had been in heterozygous condition at the start. It therefore seemed important next to obtain a method of investigation in which the effects on truncate due to the segregation of the multiple heterozygous factors that had been found should be recognizable, and could be eliminated from consideration, so that it could be determined whether or not there was any residuum of genetic variability in this character, due to actual fluctuations in the genes themselves. It seemed possible to carry out this mode of attack by a continuation of the previous main experiment, reported on pages 11 to 15 (table 7(B)), wherein the various factors had been followed out according to their chromosomes. The "residuum of variation," if there were any, should appear among the different males or females contained within any one of the separate eye-color body-color classes shown in table 7, cross 3.

The gray red males, for example, should, according to the accepted scheme of inheritance in Drosophila, all have contained identical factors for truncate, for all alike had received, without crossing over, corresponding truncate-bearing second and third chromosomes from the same heterozygous male parent. Their composition was $\frac{Y}{X}\;\frac{T_2'B}{b}\;\frac{T_3'P}{p}$ as already noted. Yet, as we have seen, some of these males appeared truncate, others intermediate, and others normal. If, then, these differences between them were really genetic, the factors for truncate, having been transmitted similarly to all of them, must have been subject to intrinsic variation. In order to determine whether the differences were genetic, the gray red males of various wing shapes needed merely to be bred to black pink females from stock; counts of the number of truncates of different grades among the offspring of the separate males would then reveal whether their character differences had a genetic basis.

The advantages of using the gray red males rather than the gray red females for the testing are threefold:—(1) a larger number of offspring can be obtained from individual males than from individual females; (2) when males are tested, since there is no crossing over between the factors for truncate and the "identifying factors," gray and red, the offspring which are gray and red like their parents furnish an ideal test of the truncate which was present in the original gray red parents, and only this class of offspring need be examined for this purpose; if female flies are tested, on the other hand, their factors for truncate become scattered more or less indiscriminately amongst all the different classes of offspring, because of crossing over. (Compare the distribution of truncate among the offspring recorded in table 7, cross 9, representing the tests of heterozygous gray red females, with the distribution among the offspring of heterozygous gray red males, shown in table 7, cross 8); (3) when males are tested, these gray red offspring, being descended substantially without recombination from their gray red fathers, may be used again for similar tests in the next generation.

Tests were therefore made of gray red males derived from crosses similar to that shown in table 7, cross 3. The results of these tests are shown in table 9, (A) and (B), the count from each male being shown on a separate line. Only those offspring of the tested males which, like the latter, are gray red, are recorded, for the reason explained in (2) of the paragraph above; the gray pinks, etc., appeared as expected, however. Table 9 (A) represents tests of males which we may call group A; these all derived their truncate-bearing chromosomes from the same heterozygous $\frac{Y}{X}\frac{T'_2B}{b}\frac{T'_3P}{p}$ father; they should therefore be genetically identical in truncation, if factors are constant. Table 9 (B)—group B—represents tests of males derived from a different father from that of group A; they may therefore be different from group A genetically. They should, however, be like one another, as they too are brothers to each other, and were derived from a single $\frac{Y}{X}\frac{T_2'B}{b}\frac{T_3'P}{p}$ father.

It will be seen, on comparing the results of the tests of the different brothers, either in group A or group B, that although there was a certain amount of "chance" variation in the proportion of truncates produced by the different males, nevertheless this variation was entirely uncorrelated with the grades of truncation in the wings of the male parents themselves. Thus, in group A, the four truncate-appearing males produced families with the following average amounts of truncation: 2.0, 2.2, 2.4, 2.6; the final average for the offspring of the truncate males therefore is *2.3+*. In the same group (A), the 3 "good intermediates" produced averages of 1.8, 2.5, and 2.8, respectively, which gives a final average of *2.5+*; the intermediates produced 2.15, 2.25, 2.4, 2.4, final average *2.3*; the 2 very slight intermediates and one normal gave 2.15, 2.47, 2.5, final average *2.4—*. In group B a similar situation exists, the families of the 2 truncates and 1 good intermediate together averaging 2.5—, those of the 3 intermediates also averaging 2.5—, and those of the 2 slight intermediates and 2 normals together averaging *2.7*. It is here evident that the variation between the families is entirely an individual matter, not connected with the class of wing shape to which the parent belongs. For there is much greater variation between the families within each parental class than between the average values for the different classes. In no case do the latter values (within either group A or B) differ by as much as 0.3 grade, in spite of the fact that the parents themselves, of these various classes, present average differences ranging from 1.0 grade (in the minimum cases) to 4.0 grades (in the maximum). Moreover, those slight differences which do exist between the offspring averages have absolutely no tendency to be of the same sign as the parental differences. It must therefore be concluded that, so far as these tests can show, the gray red brothers which were tested had been alike genetically; the relatively slight deviations in truncation, between their families of offspring, were not of genetic origin, since they were in no way connected with the variations in truncation between the brothers themselves.

Besides the above tests of males, similar but less extensive tests of sister gray red females were also carried on, which had a composition similar to that of the males, but contained in addition heterozygous T_1'. These tests yielded results identical in kind with those of the males, except that the counts were smaller, and less critical in nature, as explained on p. 21. In these tests too there was a conspicuous lack of correlation

Table 9

Tests of brother gray red males $\left(\frac{Y}{X}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}\right)$ *by black pink females.*

All tested males within a single group (A or B) are derived from a single father of same type as themselves by black pink mother. Only their gray red offspring are recorded; the numbers not in parenthesis give the total males and females, those in parenthesis give the females.

(A) (Group A)

Grade of truncation of tested male parent	Average grade of truncation of offspring	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal
4 (truncate)...	2.0	20(20)	9(9)	32(13)	41(0)	2(0)
4 (truncate)...	2.2	22(21)	11(9)	22(9)	32(1)	1(0)
4 (truncate)...	2.4	12(12)	8(8)	6(4)	15(0)	0(0)
4 (truncate)...	2.6	51(49)	24(16)	56(12)	33(0)	0(0)
Total of truncates (4)....	2.34	105(102)	52(42)	116(38)	121(1)	3(0)
3 (good intermediate	1.8	5(5)	6(6)	17(11)	20(1)	2(0)
3 (good intermediate	2.5	17(15)	10(8)	18(4)	16(0)	0(0)
3 (good intermediate	2.8	52(45)	26(17)	59(9)	11(0)	0(0)
Total of good intermediate (3)	2.53	74(65)	42(31)	94(23)	47(1)	2(0)
2 (intermediate)	2.15	14(14)	6(5)	24(14)	22(0)	1(0)
2 (intermediate)	2.25	20(15)	5(4)	16(12)	28(0)	0(0)
2 (intermediate)	2.4	20(20)	11(10)	34(17)	17(1)	0(0)
2 (intermediate)	2.4	25(24)	11(8)	17(9)	28(0)	2(0)
Total intermediates (2)....	2.30	79(73)	33(27)	91(52)	95(1)	3(0)
0.5 (very slight intermediate)	2.15	14(14)	7(7)	25(11)	19(1)	3(0)
0.5 (*very* slight intermediate)	2.5	29(22)	18(15)	50(25)	20(0)	0(0)
0.0 (normal)...	2.5	15(15)	9(7)	15(3)	9(0)	3(0)
Total of slight intermediate and normal (0.3).......	2.38	58(51)	34(29)	90(39)	48(1)	6(0)

TABLE 9 (continued)

(B) (Group B)

Grade of truncation of tested male parent	Average grade of truncation offspring	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal
4 (truncate)...	2.5	26(22)	12(10)	31(15)	21(0)	0(0)
4 (truncate)...	2.5	6(6)	2(2)	11(9)	3(0)	0(0)
3 (good intermediate).....	2.4	29(23)	12(5)	32(17)	23(0)	3(0)
Total truncate and good intermediate (3.7)	2.45	61(51)	26(17)	74(41)	47(0)	3(0)
2(intermediate)	2.3	4(4)	1(1)	7(4)	4(0)	0(0)
2(intermediate)	2.4	24(24)	14(12)	28(15)	19(0)	3(0)
2(intermediate)	2.5	23(23)	13(11)	36(6)	14(1)	0(0)
Total (intermediate (2.)...	2.46	51(51)	28(24)	71(25)	37(1)	3(0)
1 (slight intermediate)	2.1	4(4)	2(2)	8(8)	8(3)	0(0)
1 (slight intermediate)	2.55	12(12)	8(7)	10(5)	10(0)	0(0)
0 (normal)....	2.9	18(18)	8(7)	13(3)	4(0)	0(0)
0 (normal)....	3.0	21(14)	3(2)	8(2)	7(1)	0(0)
Total slight and normal (0.5).	2.71	55(48)	21(18)	39(18)	29(4)	0(0)

between the grades of the parents and those of the offspring,—the truncate females (grade 4) yielded an average offspring grade of 2.3—, among their 147 gray red offspring; their normal sisters (grade 0) yielded a grade of 2.24, among their 107 gray red offspring.

On the basis of the various tests of sibs, then, the conclusion seemed practically inevitable that the variation in the truncate character, aside from that due to segregation of multiple heterozygous factors, is entirely "somatic," and not due to fluctuations in the genes.

b. *The "pure-line" experiment*

It was thought desirable to carry the tests of factor constancy further, however. For it might have been contended that the genetic variations in any one generation may be rather small, and as such inappreciable, but that they would have a chance to become evident if allowed to accumulate through several generations of selection. A scheme of selection was therefore undertaken, with this question in view. The selection had to be carried on in such a way, however, as to exclude the possibility of effects due to recombination and the sorting out of those combinations that tended in the direction of selection. This is usually the insuperable difficulty in the way of significant selection experiments in sexually reproducing, cross-breeding organisms; it is the flaw which invalidated the early conclusions on hooded rats, for example, and other similar experiments, in so far as their bearing on the question of factor variability is concerned. What is needed in such an experiment is the prevention of effective recombination (recombination which might have an effect on the progress of the selection experiment). This is the essential feature in the pure-line idea of Johannsen. But it is not really necessary for this purpose that the organisms be homozygous and self-fertilizing, as in Johannsen's definition of the pure line, or—what amounts to the same thing—that they be inbred until, according to the probability ratios worked out by JENNINGS, they have become homozygous. Neither is the only other alternative that they reproduce asexually, as in the work of JENNINGS and of a number of other investigators. There is a third method of pure-line work, which we wish to present here, that may be used in cross-breeding organisms like Drosophila, in which all the linkage groups are known. This method consists in exposing to view completely, in one parent at least, the process of recombination, by making use of "identifying factors," and then picking out, generation after generation, the same combination for selection.[5]

This process is really, in essential principles, the one which has been already employed in the experiments just reported, where gray red flies having the same chromosome combinations were produced, and those with different grades of truncation were "selected" and bred, to find out whether they were genetically different. Here, however, the "selection" was carried on through only one generation. But in the cases where the gray red parents were males, it was possible to continue the selection further. For in these cases the gray red male offspring received (without crossing over) precisely the same paternal combination as their fathers had contained, and so they could be used for similar test crosses with black pink females from the stock bottle. As the latter crosses likewise produce offspring of the same type, these may again be used, and so, generation after generation, the selection might be continued indefinitely, by mating the gray red males to black pink females from stock. Such an experiment was accordingly undertaken with these flies.

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[5] A useful, but not so efficient, modification of this is to carry on the plus and minus selection lines without reference to recombination, and then, at the end of the experiment, to "purify" the lines, or bring them to a common basis so far as modifying factors may be concerned, by means of crosses with a common stock containing the identifying factors. If there is any effect of selection still noticeable after the "purification," it must have been in the factor studied. This method is not so effective as that above, however, for the reason that it reduces the rigor of the selection with respect to possible variations in the factor under consideration, to have other variations, indistinguishable from these, occurring at the same time, which are due to recombinations of modifying factors.

It will be desirable, before examining the results, first to analyze the conditions of this selection experiment a little more closely. The original gray red males, that were to be tested, contained the chromosome combination $\frac{Y}{X}\,\frac{T_2'B}{b}\,\frac{T_3'P}{p}$. Of this combination, they transmit their Y, $T_2'B$, and $T_3'P$ chromosomes, without crossing over, to their gray red male offspring. The entire paternally derived equipment of the offspring is therefore a direct and unalloyed continuation of the paternal side of their father, at least so far as the first three chromosomes are concerned. The maternally derived chromosomes of the offspring, on the other hand—the X-, b-, and p-containing chromosomes—are not descended from the similar chromosomes of the father, but are received directly from the black pink stock, which was used as female parent. Thus these maternal chromosomes in the offspring have a chance of being slightly different from those which the gray red father contained, although they are really derived from the same black pink stock as those in the father. In the same way, when this cross is repeated in each generation the same paternal Y, $T_2'B$, and $T_3'P$ chromosomes are continuously handed down, but the X-, b-, and p-containing chromosomes of the father are always lost in the subsequent generation and a new lot of them is received from the black pink stock. It might at first sight appear as though this polygenetic origin of the maternal chromosomes might invalidate the pure-line experiment. However, all tests have indicated that the black pink stock is sensibly homozygous, so it is improbable that differences in these maternal chromosomes would appreciably influence the characters of the offspring and thus interfere with the selection work. And much more important in this connection is the fact that since these chromosomes are discarded and renewed *at random* from the stock in each generation, any selection of factors in them (supposing factor differences do exist) can have no constant or cumulative effect on the progress of the selection. Any final and sustained differences in the effects of selection, on lines differently selected, would hence have to be due to changes which took place in that part of the germ plasm that has been handed down continuously in the selected lines—namely, the Y, $T_2' B$, $T_3' P$ chromosomes.

The above conclusion, it should be noted, implies the assumption that there are no factor-differences for truncate, existing outside the three large chromosomes. For only the first three chromosomes are under watch in the present experiment, and while these are being handed down without recombination, as in a pure line, it must be remembered that any heterozygous factors in the fourth chromosome or elsewhere (i.e., in the cytoplasm) would have a chance of being sorted out and so influencing the final result of selection. The fourth chromosome could have been watched in the same way as the first three by crossing the flies to bpb_e stock instead of to *bp*, and always choosing the gray red straight-winged male offspring as parents. If this had been done (as it was, in fact, for one generation) the transmission of the entire chromosomal genetic complex would then have been exposed to view and under control continuously. It was thought unnecessary to keep watch on the fourth chromosome, however, both because it is extremely small and also because, as has been previously mentioned, other tests had indicated there were no modifiers for truncate located there. It should, besides, be noted that such disregard of the fourth chromosome in the experiments could not invalidate the significance of a negative result. For, in case of a negative result (i.e., finding that there was no effect from selection), it would be proved, not only, (1) that all factors for truncate that had been included in the pure-line system (i.e., those located in the first three chromosomes, that had not been permitted to undergo recombination) had remained sensibly constant, but also, (2) that no factor-differences affecting truncate had *existed* outside this system,—in loci that had been allowed to undergo recombination. For if the latter factor-differences had existed, selection would have been effective by sorting them out. The same conclusion would apply both to factors in the fourth chromosome and to any supposititious factors (factor-differences) affecting truncate, that might be imagined to exist outside any of the four accepted chromosomes or linkage groups of Drosophila—that is, a negative result of the selection experiment would prove them non-existent.

The selection experiment was carried out in two lines—a "minus" or "low" line, and a "plus" or "high" line. The two lines were of course both derived from the same gray red ancestral male of composition $\frac{T_2' B}{b} \frac{T_3' P}{p}$. The count from this male (crossed by black pink female) is shown in table 10 (A), which gives as usual, only the gray red offspring of the cross. The average grade of truncation of these flies was 2.3+. One of the slight-intermediate males of this family was then chosen as the start of the low line, and one of the truncates for the high line, each being crossed to black pink females, according to the scheme explained above. The low line was then carried through twelve generations of selection, in each of which several gray red males showing the nearest approach to normal wings were chosen for parentage, and bred in separate bottles with black pink females from stock. In cases where there were conspicuous differences in truncation, between the families produced by these several males, that family giving the lowest grade of gray red male offspring was used to furnish the male parents of the next generation. The condition of this line after twelve generations of "minus selection" is shown in table 10 (B), which gives the distribution of truncation among the gray red offspring of the seven least-truncated males of this generation. It will be seen that the average grade of truncation for all these families together is 2.2+. The extremely slight difference from the original value, 2.3, is in the direction of selection, but in view of the much larger differences between families of the same line and generation, this figure must be considered as in extremely close agreement with the first finding.

The high line was carried on for thirteen generations. The method used was the same as in the other line, but of course the gray red males selected in this case were always of the highest grade of truncation available, instead of the lowest. It was found, as was to be expected, that in many cases no really high grade males were produced which could be used as parents in this line, owing to the sex-limitation of truncate and the fact that T_1' was not present; nevertheless the males chosen represented the highest grade of truncate that was being produced, and averaged about a grade higher than the males used as parents in the low

TABLE 10

Results of selection in the absence of recombination.
Only gray red offspring are recorded; total males and females not in parentheses, females in parentheses.

(A) Count from first cross of single gray red truncate male $(\frac{Y}{X}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p})$ by black pink females, from which the two selection lines were derived.

Truncate	Good intermediate	Intermediate	Slight intermediate	Normal	Total	Average truncation
11(9)	5(5)	25(12)	10(0)	0(0)	51	2.33

(B) Counts from similar crosses of males of the "low line" after twelve generations of "minus selection."

Grade of tested male parent	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal	Total	Average truncation
0 (normal)	28(28)	37(37)	30(28)	43(3)	27(0)	165	2.0
1 (slight intermediate)	29(29)	27(26)	32(15)	52(0)	16(0)	156	2.0
1 (slight intermediate)	23(23)	5(5)	20(6)	36(1)	0(0)	84	2.2
1 (slight intermediate)	26(25)	22(21)	32(10)	25(0)	0(0)	105	2.5
1 (slight intermediate)	12(12)	5(4)	11(0)	7(0)	0(0)	35	2.6
1 (slight intermediate)	17(16)	5(5)	4(1)	10(0)	0(0)	36	2.8
1 (slight intermediate)	10(9)	3(3)	8(3)	3(0)	0(0)	24	2.8
Total	145(142)	104(101)	137(63)	176(4)	43(0)	605	2.22

(C) Counts from similar crosses of males of the "high line" after thirteen generations of "plus selection."

Grade of tested male parents	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal	Total	Average truncation
2 (intermediate)	9(9)	7(6)	30(18)	21(1)	1(0)	68	2.0
2 (intermediate)	8(7)	28(26)	49(24)	23(1)	0(0)	108	2.2
2 (intermediate)	19(15)	22(17)	45(19)	40(2)	0(0)	126	2.2
2 (intermediate)	14(14)	27(25)	25(13)	28(2)	0(0)	94	2.3
Total	50(45)	84(74)	149(74)	112(6)	1(0)	396	2.18

line. The condition of this line in the last generation, after thirteen generations of "plus selection," is shown in table 10 (C). Here there is close agreement between the grades of truncation in the different families, and the average of all turns out to be 2.2— There is absolutely no genetic difference, then, between this line and the low line, in spite of a dozen generations of divergent selection. Neither of them, moreover, departs significantly from the original value. This same agreement in genetic behavior was shown moreover not only in the final generations of the two lines, but throughout their entire history. We must accordingly draw the conclusion that the truncate character, usually so plastic and so very responsive to the touch of selection both in crosses and in inbred stock, and capable as it is of existing in all degrees of "potency," nevertheless remains absolutely unaltered by selection when all recombinations of its multiple heterozygous factors is rigidly prevented, in a form of pure-line experiment which enables the distribution of practically the entire chromosomal germ splasm to be placed under observation. In other words, the actual factors or genes for truncate, lying in the chromosomes, each had a definite value, and remained constant. The experiment at the same time demonstrates that no inheritance other than chromosomal is taking place, even in the case of a character genetically so complex and so elusive as truncate. That its inheritance was entirely confined to the chromosomal linkage groups—in this case, indeed, to the first three chromosomes—is proved by the fact that there were no hereditary modifications of the character possible, so long as only these chromosomal contributions remained the same.

Thirdly, the experiment disposes of the supposition, which certain geneticists might have advanced, that the factors for truncate may in heterozygous individuals be weakened, or undergo contamination by their normal allelomorphs. For here the truncate factors of both lines were kept heterozygous for twelve generations without their value or "potency" being affected.

THE CAUSE OF THE INCONSTANCY OF TRUNCATE STOCK

a. *Proof of the viability and non-sterility of flies homozygous for the intensifiers*

The question still remained unanswered, then, why had all the attempts to obtain genetically constant truncate stock ended in failure. Since the factors themselves were constant, and the stocks were inconstant, genetically, it followed that all the stocks must be, for some reason, heterozygous in one or more of the factors for truncate. This means that the homozygote, of one sex at least, must be either sterile, or nonviable, or else incapable of being formed at all. It was evident, however that this condition did not apply to all the factors for truncate, otherwise it would be possible, by out-crossing to normal, to obtain heterozygous truncates that were somatically and genetically of as high a grade of truncation as the best truncate flies of the stock bottle. Nearly all the truncates resulting from out-crosses were, on the contrary, of conspicuously lower grade than their truncate parents. And it could be shown, more specifically, that the factor T_1' was not subject to this limitation. For, in the first place, T_1' could exist in fertile truncate males. This had been proved by the reciprocal crosses considered on p. 15 and 16. And as males which carry a given sex-linked factor in their single X chromosome are usually of a similar type, somatically, to the females homozygous for that factor, it was fairly certain that the females homozygous for T_1' were viable also. It was less certain that they were fertile, on account of the difference between the reproductive systems of male and female. The viability and fertility of females homozygous for T_1' were, however, independently proved by the fact that the daughters of the out-crossed truncate females were, on the average, just as frequently truncate, and of just as high a grade of truncate as the daughters out of the out-crossed truncate males (see page 16, and table 6). The males necessarily transmitted T_1' to all of their daughters; the females therefore must have done the same, and so must have been homozygous for T_1'.

It was also clear that not only T_1' but in addition either T_2' or T_3' (or, if they be compound, then one or more of the factors contained in them) could exist with some degree of fertility in homozygous condition. For the truncate or intermediate T_1' males derived from out-crosses of truncate females were usually of much lower grade than the truncate males of truncate stock. Since both alike might possess T_1' in their single X chromosome these differences in truncation were evidently due to the heterozygosis of the F_1 males in respect to some non-sex-linked factors (T_2' or T_3' or some component of them), for which the stock males were homozygous.

Crosses were then made to secure evidence, in the case of the two latter factors, as to whether or not they could exist in homozygous condition. For this purpose recourse was again had to crosses of single heterozygous gray red males $\frac{T_1'X}{Y}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ by stock black pink females. One of these crosses is shown in table 11 (A); it is identical in nature with that of table 7, cross 3. From the offspring of this cross, flies were chosen for five parallel crosses, the results of which were to be compared.

The first three of these crosses were concerned with the question of homozygosis in respect to T_3'. The first cross was of a slight-inter-

TABLE II

Crosses to test the possibility of securing homozygous T_2' and T_2'.

(A) Preliminary back-cross of heterozygous truncate male to black pink stock

Nature of parents ♀	♂	sh T	Gray red T	Gray red I	Gray red S	Gray red N	Gray pink T	Gray pink I	Gray pink S	Gray pink N	Black red T	Black red I	Black red S	Black red N	Black pink T	Black pink I	Black pink S	Black pink N
$-\ \frac{b}{b}\ \frac{p}{p}$ Black pink long	$\frac{T_1'}{}\ \frac{T_2'B}{b}\ \frac{T_2'P}{p}$ Gray red truncate		18 (0)	9 (4)	16 (16)	3 (3)			18 (3)	38 (17)				47 (21)				48 (23)

(B) Crosses of flies derived from (A)

		sh T	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
$\frac{T_1'}{}\ \frac{b}{b}\ \frac{p}{p}$ Black pink long	$\frac{T_2'B}{b}\ \frac{T_2'P}{p}$ Gray red slight		10 (6)	18 (11)	11 (0)	3 (0)		8 (4)	14 (10)	19 (6)				40 (22)				41 (23)
$\frac{T_1'}{}\ \frac{b}{b}\ \frac{T_2'P}{p}$ Black red long	$\frac{T_2'B}{b}\ \frac{p}{p}$ Gray pink long		5 (2)	20 (11)	14 (6)	5 (1)		8 (7)	20 (13)	23 (11)				34 (14)				43 (20)
$\frac{T_1'}{}\ \frac{b}{b}\ \frac{T_2'P}{p}$ Black red long	$\frac{T_2'B}{b}\ \frac{T_2'P}{p}$ Gray red slight.	11 (8)	7 (4)	4 (2)	7 (3)	1 (0)		3 (2)	3 (1)	5 (2)				24 (15)				7 (3)
$\frac{T_1'}{}\ \frac{T_2'B}{b}\ \frac{p}{p}$ Gray pink slight	$\frac{b}{b}\ \frac{T_2'P}{p}$ Black red long			5 (3)	2 (1)	5 (2)			4 (4)	4 (3)		2 (1)	1 (1)	7 (5)				17 (8)
$\frac{T_1'}{}\ \frac{T_2'B}{b}\ \frac{p}{p}$ Gray pink long	$\frac{T_2'B}{b}\ \frac{T_2'P}{p}$ Gray red slight	1 (1)	5 (3)	8 (4)	4 (2)	7 (3)		1 (0)	9 (4)	13 (7)		5 (2)		16 (8)			2 (1)	11 (3)

(The number of males is given in parenthesis under the number of flies of both sexes.)

mediate gray red male (composition $\frac{X}{Y}\ \frac{T_2'B}{b}\ \frac{T_2'P}{p}$) by one of its black pink sisters ($\frac{T_1'X}{X}\ \frac{b}{b}\ \frac{p}{p}$) derived from the same bottle. This cross is of the same type as the parental one of table 11 (A) except that T_1' is distributed to half of the daughters and to half of the sons, instead of to all of the daughters and none of the sons. The count, given on line 1, table 11 (B), shows the results to be expected on the basis of the parental compositions, there being the usual four classes, gray reds, gray pinks, black reds, and black pinks, in remarkably equal numbers. Only the grays are truncated, and the gray reds especially, showing a count of 10 truncate: 18 intermediate: 11 slight-intermediate: 3 normal. ("Good intermediates" and "intermediates" are classed together in this and the following records.)

The second cross was like the first, except that the female was heterozygous for $T_3'\,P$, instead of the male. This cross therefore had the formula $\frac{X}{Y}\,\frac{T_2'B}{b}\,\frac{p}{p}$ (long-winged gray pink male) by $\frac{T'X}{X}\,\frac{b}{b}\,\frac{T_3'P}{p}$ (long black red female). It will be seen that the results, shown on line 2 of table 11 (B) are very similar in character to those of the first cross, except that there may be a slight decrease in the proportion of high-grade truncates among the gray reds, as compared with the first cross. This change, if not a chance deviation, is due to the crossing over between T_3' and P, which was possible in the female.

These first two crosses really served as controls for the third cross, which was concerned with the possibility of securing homozygous T_3'. The third cross was really a combination of the first and second, in that both the male and female parents were heterozygous for $T_3'\,P$, instead of the male alone, as in cross 1, or the female alone, as in cross 2. The parents, then, were a slight-intermediate gray red male which was a brother of the similar one used in the first cross and one of its black red sisters which was also a sister of the similar female that was used as a parent in the second cross. The formula for the cross is $\frac{X}{Y}\,\frac{T_2'B}{b}\,\frac{T_3'P}{p}$ by $\frac{T_1'X}{X}\,\frac{b}{b}\,\frac{T_3'P}{p}$. Here, since both parents are heterozygous for $T_3'\,P$, the reds should bear the ratio of 3 : 1 to the pinks, instead of 1 : 1, as before, and some of the reds,—one-third or fewer of them, depending on the exact amount of crossing over between T_3' and P,—will be homozygous for T_3', provided T_3' can exist in homozygous condition. This homozygosis in T_3', if it can exist, might then be reflected in a more strongly truncated condition of the flies of the gray red classes; if, on the other hand, homozygous T_3' cannot exist, the gray red flies will not, on the average, be more markedly truncated than those of the previous cross. They would, if anything, average less truncate than in cross 1, because of the possibility here of crossing over between T_3' and P (red). Moreover, the ratio of reds to pink would be 2 : 1, or at least less than 3 : 1, if the reds homozygous for T_3' failed to appear. The count is shown on line 3 of table 11 (B). The ratio of reds to pinks is exactly 3 :1 (54 : 18), as it should be if the homozygous T_3' live. Examining the gray reds, it is clear that there is among them a new class of individuals, with respect to truncate, that is never found in conspicuous numbers in out-crosses. This has been called "short-truncate"; it is of the same grade as the highly-truncated individuals of selected truncate stock. Almost exactly one-third of the gray red individuals belong to this grade. (It will be noted that the gray pinks, on the other hand, are no more truncated than before, there being no special environic conditions here favoring truncation.) It is therefore evident that the whole chromosome containing T_3' is capable of existing in homozygous condition, and that, provided T_1' and T_2' are present as heterozygous factors, then homozygosis in T_3' alone is necessary in order to produce an average grade of truncation equal to that of the best selected inbred stock. It was found, on attempting to breed the short-truncates produced in this experiment, that although the flies were not sterile, yet the infertility (of the females especially) was so great as to make it extremely difficult to perpetuate the line, just as in the case of the "best" inbred truncate stock. Crosses of the same type as that of table 11 (B), cross 3, have been repeated several times, and exactly similar results, including the production of a conspicuous class of short-truncates, have always been obtained.

The first, fourth and fifth crosses were concerned with homozygosis in respect to T_2'. The fourth cross was similar to the first except in that the female was heterozygous for T_2' instead of the male, so that here there could be crossing over between T_2' and B.. The formula is $\frac{X}{Y}\ \frac{b}{b}\ \frac{T_3'P}{p}$ by $\frac{T_1'X}{X}\ \frac{T_2'B}{b}\ \frac{p}{p}$ (long black red male by slight-intermediate gray pink female). The parents were sibs of the parents used in the other crosses. This cross accomplishes the same purpose for T_2' that the second cross did for T_3'. The very small count, given on line 4 of table 11 (B) is nevertheless sufficient to show that the general character of the cross is like that of the first, except in that the grays here include fewer truncates, and that some of the blacks are truncated, owing to the crossing over between T_2' and B in the female. The first and fourth cross served as controls for the fifth, in which opportunity was given for T_2' to become homozygous. In the fifth cross, both male and female (sibs of the previous parents) were heterozygous for $T_2' B$, instead of just the male, as in cross one, or just the female, as in cross four. This time, then, a slight-intermediate gray red male like that in the first cross was mated to a gray pink sister like that in the fourth cross ($\frac{X}{Y} \frac{T_2' B}{b} \frac{T_2' P}{p}$ by $\frac{T_1' X}{X} \frac{T_2' B}{b} \frac{p}{p}$). In this case, as both parents contain heterozygous $T_2' B$, the expected ratio of grays to blacks would be 3 : 1, instead of 1 : 1, as in the other crosses, and one-third or fewer of these grays (both reds and pinks) should be homozygous for T_2' and might therefore be more truncated than the corresponding classes of grays (red or pink) in the first cross. But if the grays homozygous for T_2' fail to appear, this 3 : 1 ratio should be reduced towards 2 : 1, and the grays in this cross should not be more truncated than those in the first cross. The count, on line 3, table 14, shows 48 grays to 34 blacks. This is nearer 2 : 1 than any other simple ratio possible here and certainly it could scarcely be taken for a 3 : 1 ratio. The gray reds, although including one short-truncate, such as appear in isolated instances in outcrosses to black, are not on the whole significantly more truncated than those in the first cross; the gray pinks, which are just as useful for the present purpose, are, if anything, less truncated than in the first cross, and do not include any flies that have a higher grade of truncation than that found among the gray pinks there. The two most truncated gray red males, moreover, were tested by crossing to black pink, and both proved to be heterozygous for T_2', giving both gray truncate and black long-winged offspring, and no black truncates. The evidence of these experiments hence indicates that T_2' cannot become homozygous. Yet it is not conclusive, for there is a possibility that homozygous T_2' is no more truncate in appearance than heterozygous T_2'. The deficiency in the number of grays, also, might be explained away, by supposing that special circumstances made all the grays here relatively less viable than the blacks. It should be mentioned, however, that crosses one and five were later repeated, with results similar to those of the present experiments: the ratio of grays to blacks in the repetition of experiment five was 61 : 32, and the truncate averaged of no better grade there than in the control cross corresponding to cross one (no short-truncates at all were produced this time).

b. *Experiments to test the possibility of securing homozygous T_2'*

Other crosses were then undertaken, which might determine more definitely whether the T_2' homozygote could occur. It would not have been feasible here to test flies for homozygosis in the ordinary simple manner,—that is, merely by finding the total proportion of truncates thrown, because the proportion of truncates is subject to modification in so many ways. A way was therefore found of "marking" the T_2' factors, by binding them to identifying factors (B and b), so that it could be determined in the tests whether a fly had only one T_2' factor, or two T_2' factors differently "marked", as will be explained presently. For this purpose, black truncate flies were secured that had arisen as crossovers in a cross similar to number four above (see line 4, table II (B). From these a stock of black truncate (inconstant for truncate, of course) was obtained. Black truncates (presumably heterozygotes containing $\frac{T_2'\,b}{b}$) were then mated to heterozygous gray truncates having the second chromosome composition $\frac{T_2'\,B}{b}$. Both reciprocal crosses were made. From these crosses gray truncate offspring,—usually the male offspring,—were chosen, and tested by crossing to black long-winged stock.

In the cross of $\frac{T_2'\,b}{b}$ males by $\frac{T_2'\,B}{b}$ females, the gray truncate male offspring that were tested may have received either a crossover gray chromosome,—B,—or a non-crossover gray chromosome,—$T_2'\,B$,—from their mother. In the former case, which would be less frequent, they must have received $T_2'\,b$ from their father, since they showed the truncate character; their composition, then, would be $\frac{B}{T_2'\,b}$. They would reveal this composition, in the test crosses with black long, by producing gray offspring $(\frac{B}{b})$ all long-winged, and black offspring $(\frac{T_2'\,b}{b})$ some of which were more or less truncated. (Enough intensifiers were present to insure the visibility of the truncate in most of the flies carrying T_2'.) Of the fifteen gray truncate males tested, four proved to have this composition, as they threw black truncates, but no gray truncates. Here there was no chance for homozygosis of T_2'. The other eleven gray truncate males proved to have received $T_2' B$ from their mother, for they all gave rise to some gray truncates on crossing to black long. It was to be expected, if T_2' can be homozygous, that half of these eleven males would have received the $T_2' b$ chromosome from their father, the rest merely the b. The former, then, would throw black truncates, the latter only black longs, in addition to the gray truncates. As a matter of fact none of these flies which threw gray truncates threw black truncates, only longs appeared within the black class of offspring. The composition of all eleven must therefore have been $\frac{T_2' B}{b}$, although, if T_2' can occur in homozygous condition, half of them should have been $\frac{T_2' B}{T_2' b}$.

From the reciprocal cross, of $\frac{T_2' B}{b}$ male by $\frac{T_2' b}{b}$ female, 10 gray truncate male offspring were tested. In this cross, there will be no $\frac{B}{T_2' b}$ class, for all the gray offspring must receive $T_2' B$ from their father, since there is no crossing over between T_2' and B in the male. Correspondingly, it was found that all the (gray) males tested threw gray truncates. Half of these males, as before, may have received b from their other parent, and the rest $T_2' b$. But, as before, it was found that all the black offspring were longs; here again, then, all the flies had been heterozygous, $\frac{T_2' B}{b}$, although there had been a chance for the half of them to have been homozygous, $\frac{T_2' B}{T_2' b}$. Five of the gray truncate female offspring of this cross were also tested, by crossing with black long males; similar considerations apply to them, except that a heterozygous $\frac{T_2' B}{b}$ female would throw some black truncate offspring by crossing over; it would not throw nearly as many black truncates as gray truncates, however, and so it could be distinguished from the homozygous $\frac{T_2' B}{T_2' b}$ female. It was found that all five females were of the heterozygous type, $\frac{T_2' B}{b}$, just as in the case of the males.

Another cross, similar in principle, involved the dominant identifying factor star, in chromosome II, as well as black. A black pink truncate fly (obtained by crossing and subsequent selection) was crossed to star pink. A son showing the characters star, truncate and pink,—composition $\frac{T_2' \ b}{S' \ B} \ \frac{p}{p}$, —was then crossed by a short-truncate female from the inbred truncate stock. Forty-six male offspring which did not have star eyes, and which had therefore received $T_2' \ b$ instead of $S' \ B$ from their father, were then tested by back-crossing them separately to non-truncate black pink females. The composition of these males must have been $\frac{Y}{T_1' X} \ \frac{T_2' \quad b}{T_2'(?)B} \ \frac{p}{T_3' P}$; the question was whether any of them had received $T_2' \ B$ from their truncate mother, in addition to the $T_2' \ b$ that they must have gotten from their father. If T_2' can be homozygous, at least half should have received $T_2' \ B$, and should therefore give rise to gray truncate offspring as well as black truncates. All forty-six, however, failed to produce any gray truncates, although they all threw black truncates in very high percentage, particularly among the red female offspring, which contained T_3' and T_1'. The original short-truncate female from selected stock must therefore have been heterozygous for T_2', and when her eggs were fertilized by the T_2' b-bearing sperm, only those which had failed to receive the maternal T_2' developed. Adding these 46 to the previous 26 tests, there is a total of 72 flies, at least one-half of which should have been homozygous for T_2', had this been possible, but all of which were found to be heterozygous.

We may therefore conclude with certainty that homozygous T_2' cannot exist. This explains not only why there was such difficulty met with in the attempt to get pure truncate stock, but also why truncate, even when it acted as a dominant in crosses with black, never gave more than half of the F_1 showing truncate (see tables 6 and 7). Twin hybrids, truncate and long, were produced, as in out-crosses of Oenothera, Matthiola, and beaded Drosophila (see page 41), half of them in the present case receiving the T_2' chromosome and half the t_2'. Tests of such F_1 longs, moreover, showed many of them to be genetically long, and not merely T_2'-carrying flies that lacked intensifiers or happened to vary somatically in the direction of normal (although of course, some of the longs were of this type, in cases where more than half of the F_1 appeared long). Table 12, lines 1 and 2, shows the result of such a test of an F_1 long male which had arisen from an out-cross of a short-truncate female of inbred stock by a black pink male. When this long was back-crossed to black pink, many of the gray red offspring should have appeared truncate, had the F_1 carried T_2', but, as the table shows, not one truncate appeared. One of the slight intermediate F_1 males, on the other hand, which was a brother of the long, gave the count on the second line, when tested similarly. Thus, even the short-truncate of inbred stock is heterozygous for T_2', and gives the results expected of a heterozygote, on out-crossing. It may be noted that the tests of these normal-appearing F_1 males are in striking contrast to the results obtained by crossing normal-appearing males from a back-cross like that in table 7, cross 3, where the identifying factors show that the flies, though normal, must really carry the factors for truncate.

TABLE 12

Tests of F_1 males from a cross of short truncate female by black pink male. F_1 males tested by back-crossing to black pink.

Character of F_1 male tested	Gray red				Gray pink				Black red				Black pink			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
Normal	0	0	0	21	0	0	0	25	0	0	0	23	0	0	0	26
Slight intermediate	0	3	3	2	0	0	2	14	0	0	0	17	0	0	0	11

c. *The reason for the non-appearance of homozygous T_2'*

The failure of T_2' to appear in homozygous combination must be due either to the fact that it acts, when homozygous, as a lethal factor, or that there is a selective fertilization of T_2' eggs by t_2' sperm exclusively. In the latter case, when heterozygous $\frac{T_2'\,B}{b}$ flies were crossed with each other, a ratio of 3 grays $(\frac{T_2'\,B}{b})$: 1 black $(\frac{b}{b})$ should be produced, since all $T_2'\,B$ eggs would be fertilized by b sperm, and half the b eggs would be fertilized by $T_2'\,B$ sperm. In the case of a lethal factor, on the other hand, only 2 grays : 1 black should appear, for one-third of the grays would consist of the homozygous $\frac{T_2'\,B}{T_2'\,B}$ combination, that would die. We have seen (page 35) that the 2 : 1 ratio is actually obtained; thus T_2' is a lethal, like yellow in mice, and like most dominant mutant factors, in Drosophila at any rate.

d. *The cause of the infrequency of normals in the selected stock*

This "yellow-mouse" hypothesis had of course often been considered, as a possible explanation of why stocks of truncate were always genetically inconstant, but it had not previously been thought probable, because the highly selected, inbred truncate threw only 10 percent of normals (and many of these, even, carried T_2') whereas yellow mice throw as much as 33⅓ percent of normals (or non-yellows). A cross of $\frac{T_2'}{t_2'}$ by $\frac{T_2'}{t_2'}$ likewise should give 2 $\frac{T_2'}{t_2'}$ (more or less truncated) : 1 $\frac{t_2'}{t_2'}$ (long), provided the $\frac{T_2'}{T_2'}$ die. Either, then, in the case of the selected truncate, most of the $\frac{t_2'}{t_2'}$ which are formed die also, owing to the existence of another lethal or semi-lethal factor in the chromosome with t_2', or, if they live, many of them must appear truncate, like the $\frac{T_2'}{t_2'}$ flies, owing to some other chief factor for truncate that they may contain. This other factor would have to lie in the second chromosome, for we have seen that flies containing other chromosomes of truncate stock, but not having the second so derived (the black red flies of table 7, cross 3), even when inbred, cannot give rise to truncates (table 7, cross 5). It would, moreover, have to be a recessive, for we have seen that heterozygous flies which fail to receive T_2' are never truncate, even if one of their second chromosomes is derived from truncate stock (p. 39).

A test was therefore undertaken, to determine whether there existed, in the t_2'-bearing second chromosome of the selected stock, a recessive factor for truncate, which, when homozygous, was able to cause the truncate character to appear, even in the absence of T_2' itself. This test was performed by inbreeding the gray red flies $\left(\frac{T_1'X \text{ or } Y}{X}\ \frac{t_2'B}{b}\ \frac{T_3'P}{p}\right)$ obtained in the last tests for homozygous T_2', reported on p. 38. All these flies had received, it will be remembered, one gray-bearing chromosome derived from a short-truncate female of the selected stock; this chromosome had been found not to contain T_2'. If, however, it contained a recessive "chief" factor for truncate, in any of the families (the chromosome was not necessarily the same in the different families, on account of the possibility of crossing over in the stock truncate fly from which it was derived), then, when the gray flies of such a family were crossed together, some flies homozygous for the recessive truncate would be produced, and many of these should show the truncate character (since the intensifiers also are present). Nineteen of these tests were made in all, the gray red offspring of each of nineteen of the males that had been tested for homozygosis being inbred to each other in mass culture, family by family; thus, if the original truncate female had been heterozygous, so that only certain families contained the factor, it would have a chance to become homozygous in these lines. None of the 19 families produced any truncates, however, and the hypothesis of a recessive "chief" factor to explain the low proportion of normals in the selected truncate stock therefore had to be discarded.

The only remaining possibility was that the truncate stock contained a lethal (or semi-lethal) factor in the second chromosome that carried t_2', for since it was shown that a $\frac{t_2'}{t_2'}$ combination in the selected stock would not give rise to truncate-winged flies, the excess of truncates (or deficiency of longs) proved that there was a real deficiency, i.e. a dying off, of the $\frac{t_2'}{t_2'}$ flies that were formed at fertilization.

A similar condition of affairs had been found by MULLER (1917) to exist in the case of the beaded-winged stock of Drosophila, and was called by him a state of "balanced lethal factors." In such a case, there are present, in homologous chromosomes, two different lethal factors, each of which kills off an opposite type of homozygote, and which, together, thus cause a heterozygous stock apparently to breed true, or nearly true. The balanced lethals in the case of the truncate stock are T_2', which kills the homozygous truncates, and the recessive lethal factor in the opposite chromosome, which kills the homozygous $\frac{t_2'}{t_2'}$ flies.

As shown in the report on the beaded case, the occurrence of such recessive lethals by mutation must be comparatively frequent. Bearing this in mind, the existence of this factor in the truncate race is easily explained, as follows: A stock having a character such as truncate, which is itself due to a lethal (T_2'), must continually throw normals. When such a character is selected, therefore, an endeavor will be made to select a line throwing as few normals as possible. In the course of time, various lethal factors will arise, in different loci, most of which will be eliminated by natural selection, but when a lethal arises in the normal homologue of the chromosome containing the factor that is being selected for (T_2'), it will cause the killing off of a large number of the undesired normals; consequently the line in which this factor has arisen will be perpetuated in the selection. The nearer the locus of this factor lies to the locus of the desired factor (T_2'), the fewer will be the normals that can escape, by crossing over, from the lethal action, and so the better will be the chance of the perpetuation of this race in the experiment. After a long course of selection, then, it is to be expected that the stock would show the presence of a lethal factor nearly opposite, in location, to the lethal factor that is being selected for, and a condition of "balanced lethals" would have arisen. For the further consequences of this condition the reader must be referred to the account of the beaded case.

e. *The source of the normals which did occur in the selected stock*

In the case of the truncate stock, it is likely that there was a certain amount of crossing over between T_2' and the recessive lethal. This would partly explain the presence of the 10 percent of normals in the selected stock, for such normals could arise from a $\frac{T_2' L}{t_2' l}$ female, from a crossover gamete $t_2' L$ (l here indicates the recessive lethal; L its normal allelomorph). Such normals would never give truncates if crossed together, as they do not contain T_2'.

Table 2 showed, however, that some of the normals thrown by the stock could give rise to truncates. Some of these normals, then, may have been merely somatic variants towards normal, which nevertheless had the full complement of truncate factors. In that case it would have to be assumed that in these experiments they had been mated to the crossover normals, which lacked T_2' and which would be indistinguishable from them somatically, for these matings gave fewer truncates than ordinary matings of somatic truncates. But it seems unlikely that this special sort of mating should be made frequently, and so it is more probable that most of these normals, which were able to throw some truncates, were T_2'-bearing flies that lacked one or more of the intensifiers. It has been explained that females homozygous for the intensifier, T_3', are extremely infertile, and since in the selected stock even those eggs which are laid are killed off in large numbers by the lethals of chromosome II, it will be readily understood that truncate stock homozygous for T_3' could not be long maintained. It was, in fact, found continually in the original selection experiment that the very high lines died out through infertility, and that the stock had to be replenished from mass cultures of somewhat lower grade. The stock consequently was probably impure for T_3' as well as T_2', even though many of the individuals in it must have been homozygous for the former factor.

THE LOCUS OF TRUNCATE

The above experiments were sufficient to demonstrate the nature of the mechanism of inheritance of the truncate character. They showed that it conformed regularly to the principles of chromosome heredity, factor constancy, etc., and disclosed the causes of the inconstancy of the inbred stock, and of the indefiniteness and variability of the ratios thrown in crosses.

Further determination of the precise number of modifying factors for truncate present in each chromosome, and the location and exact effect of each, in the various possible combinations, would have required, for the most part, elaborate tests—much more elaborate than the preceding ones—and yet these details would have been of little general significance, once it had been found out what was really "the trouble" in the truncate case. Some more exact tests were at first undertaken, with these objects in view, but many identifying factors had to be used for this purpose, in order to follow the crossing over, and it was found that some of these factors also influenced the truncate character. This circumstance, taken together with the fact that the classes containing the intensifiers far overlapped those lacking them, on account of the natural somatic variation of truncate, made a direct estimation of the percentage of crossovers impossible. An example of the confusion caused in these ways is seen in table 13, which represents a count of 196 offspring of a back-cross designed to find the locus of T_3'. A female heterozygous for truncate and for three third-chromosome identifying factors—pink eye (p), kidney eye (k), and sooty body color (e^s), was back-crossed to a pure p k e^s male. It will be seen that the truncate shows rather close linkage to P, and yet the crossover classes all show a fair amount of truncate, which is distributed among them nearly proportionally. Thus the truncate intensifier shows no closer linkage to P than to either of the other factors, and its locus is indeterminable. Such a result can be explained only by supposing that several modifiers of truncate are here concerned.

Difficulties of the above sort would not have been insuperable, as the flies from such a count could each be tested out, separately, but a much

TABLE 13

Back-cross of truncate to pink kidney sooty.

$$\left(\frac{T_1'}{\quad}\ \frac{T_2'}{\quad}\ \frac{T_3'}{pke^s}\ ♀ \times \frac{p\ k\ e^s}{p\ k\ e^s}\ ♂\right)$$

	P K E				*p k e^s*				*P k e^s*				*p K E*				*P K e^s*				*p k E*			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
♀	3	1	0	34	1	4	2	36	1	1	0	1	0	0	0	8	0	0	0	6	0	0	0	4
♂	3	4	1	30	0	4	0	32	0	0	0	4	0	1	0	2	0	1	1	7	1	1	0	2

more elaborate system of experiments would have been necessary for this purpose than the value of such information justified. There would, moreover, have been no such thing as a "complete account" of the truncate case, unless all known and unknown mutant factors of Drosophila had been tested, in all possible combinations, with respect to their effect on truncation. It was therefore decided to confine the "intrachromosome" investigation simply to a determination of the locus of T_2', the chief factor for truncate.

Certain data regarding this may be obtained from the counts of table 7(B), crosses 4, 6, and 9, where females heterozygous for truncate and black $\left(\frac{T_2'\,B}{b}\right)$ were back-crossed to black long males. Here the long-winged offspring must be disregarded, as they are genetically heterogeneous, including many flies that carry T_2 besides the really genetic longs. The percentage of crossing over may, however, be obtained from the count of those that show any degree of truncation. Among the latter, the grays are of course non-crossovers, the blacks crossovers. There are 173 flies in these three crosses showing some grade of truncation, and among them 41 are black (crossovers), the rest gray. This would make the crossing over between truncate and black $\frac{41}{173}$, or 24 percent. In various crosses of the same type 375 truncates have been counted in all, including 100 blacks; this makes the percentage of crossing over nearly 27.

Truncate was also crossed to a stock of black purple curved (all in chromosome II), and F_1 truncate females were back-crossed to $b\ p_r\ c$ males. The count of 530 individuals is shown in table 14; there is the usual percentage of crossing over between b, p_r and c (b-$p = 6$ percent, p_r-$c = 17$ percent). To find the linkage with truncate, only the 119 offspring showing truncation can be used, just as before. Among these there are 22 percent of blacks, indicating a "distance" of 22 units in this cross. This, averaged with the previous results, would place T_2' at 25.5 units from black. Double crossing over is so free in this cross, however, that it is not possible to tell with certainty whether T_2' is on the same side of black as curved is (i.e., to the "right") or on the opposite side (the "left"). Yet the fact that in crossing over between black and purple the truncate remained with the black, indicated that T_2' was probably on the opposite side of black from purple,—that is, to the "left".

A test was accordingly made to determine on which side of black T_2' lay. This was done by means of a back-cross involving truncate, black, and star. Star (S') is a dominant factor lying 46 units to the left of black, at locus 0. In the first place, a star truncate stock was secured by crossing truncate with star and selecting the crossovers in the second generation, and in the second place this star truncate was crossed to black, and one of the heterozygous female offspring, which appeared star truncate, was back-crossed to a black male (triple recessive). The count, given in table 15, shows clearly that T_2' is to the left of black, since twenty-three of the twenty-four flies known to be crossovers between truncate and black (i.e., those showing both truncate and black) are also crossovers between star and black (star, truncate, black). The exact percentages are not very significant in a count of this size (total count 130) but the order of the factors is determinable with certainty.

TABLE 14

Back-cross of truncate to black purple curved.

$$\left(\frac{T_1'}{}\ \frac{T_2'}{b\ pr\ c}\ ♀ \times \frac{b\ pr\ c}{b\ pr\ c}\ ♂\right)$$

	BPr C				*bpr c*				*Bpr c*				*bPr C*			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
♀	26	13	6	56	7	5	0	69	0	1	0	3	0	0	0	10
♂	20	12	11	83	5	1	2	99	0	0	0	4	0	0	0	8

	BPr c				*bpr C*				*Bpr C*				*bPr c*			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
♀	0	0	0	10	3	2	0	25	1	1	0	1	0	0	0	2
♂	4	0	0	10	0	0	1	30	0	0	0	1	0	0	0	0

When all these tests of truncate with black are averaged together, a

TABLE 15

Back-cross of star, truncate and black.

$$\left(\frac{T_1'}{\quad}\;\frac{S' T_2'}{\quad b}\;\frac{T_3'}{\cdot}\;\text{♀}\times\frac{b}{b}\;\frac{T_3'}{p}\;\text{♂}\right)$$

Non-crossovers		Crossover 1		Crossover 2		Double crossover 1, 2	
Star truncate	Black	Star black	Truncate	Star truncate black	Normal	Star	Truncate black
41	36	6	6	23	14	3	1

percentage of crossing over of about 27 is obtained. The distance between truncate and star, as given in the last test, is 12.3. As the percentage of separations between star and black is known to be about 39, the results of the tests of truncate with star and with black agree fairly well, and it may be concluded that truncate is between star and black, at a locus of about "12"—i.e. 12 units to the right of star, which is taken as being at 0.

ON THE MODE OF ORIGIN OF THE TRUNCATE COMPLEX

On the basis of the information that has been gained concerning the genetic basis of the truncate complex, its mode of origin may in a general way be reconstructed. It is probable that either the factor T_2' or one of the intensifiers had arisen by mutation and persisted unnoticed in the ancestral beaded line for some time before the first truncate fly was discovered. For the original truncate, it will be remembered, gave rise to a moderate proportion of truncate F_1, and to a much higher proportion of truncates in some of the F_2 families. Now, the moderate proportion of truncates in F_1 would not have been possible in the first place, had not an intensifier of truncate been present,—it is only necessary to recall here the extremely small proportion of truncates produced in F_1, in the absence of black, even when the intensifiers are present in heterozygous condition (see table 3(A), representing crosses of selected stock to wild type). Secondly, had not an intensifier been present, it would not have been possible to raise so materially the proportion of truncates in F_2, since only intensifiers, and not T_2' itself, could have been made homozygous by this process.

One possibility, then, is that T_2' had existed for some time in the stock, and suddenly burst into visibility when T_1' or T_3' arose by mutation. Before this occurrence, it would have been detectable practically only as a lethal factor, by the disturbances it produced in ratios, or, if elaborate tests were made, as a factor giving the fly a very slight "instability" of wing shape, or "tendency" to have shortened wings, which manifested itself once perhaps in 100 cases ordinarily, but much more often in the presence of the factor for black (thus recalling the alleged mode of inheritance of certain nervous defects in man). On the other hand, it is more probable that one of the intensifiers had existed first, for these would have been better able to persist in the stock than a lethal factor. The intensifier would not have been detectable at all at this time, unless, in the case of T_3', tests of fertility had been made. But, whichever of the two suppositions is true, it is evident that one of the mutant factors had arisen and persisted unobserved until the second mutation took place.

This situation raises the question as to how many such mutations—producing no visible result on the organism, but changing only the composition of the chromosomes, and, through them, the ultimate potentialities of the race—may be continually occurring unseen. Certainly the occurrence of such mutations is by no means rare, judging by the comparative frequency with which modifying factors are found, in races which happen to have the "chief" factor for that character already present. Thus, when selection is made for bar eye, bar modifiers are found; on selection for beaded wings, beaded modifiers; on selection for dichaete bristles, dichaete modifiers; all of which modifiers have little or no effect on the fly lacking the chief factor for bar, beaded, or dichaete, respectively. Yet such mutations must be occurring with similar frequency (though not selected for and artificially perpetuated) in stock lacking the specific chief factor; so, in wild stock, modifiers must be arising for bar as in the bar stock, and at the same time for beaded as in the beaded stock, for truncate as in the truncate stock, and similarly for innumerable other potential characters not actually realized in the stock at that time. Hence it may well be that there are more mutations for which no means of influencing the somatic characters exists, than visible "character-mutations," and undoubtedly there are many on the edge of visibility. Many of these "invisible" or "barely visible" mutant factors should persist in particular lines, merely as a matter of chance, and they would affect not only chromosome composition and behavior, but also the possibilities of future character-evolution for that line. Such, at least, was the case with the truncate line which, by means of the prior possession of an "invisible" factor for truncate, was enabled to produce the truncate "evolution series." Here is a situation which may give some satisfaction to believers in orthogenesis.

The truncate which had thus appeared probably contained, as yet, only one intensifier, and no balancing lethal, for it could not be increased by selection for a considerable time. When, however, each of the other contributing factors finally appeared in the course of the breeding, the line containing it was purposely selected, since it threw more and better truncate. It has been previously explained that there is nothing remarkable about the fact that a balancing lethal did arise. The fact that it was also possible to find one or more additional intensifiers may however call for some comment. This responsiveness of truncate to selection,—the appearance of intensifiers when sought for,—may be shown to be an inherent characteristic of the truncate race, dependent on a particularly high modifiability of the embryological process whereby the truncate character is produced. The degree of modifiability of the developmental reaction that causes any given character may be estimated by at least two criteria: (1) by the somatic variability of the character among individuals of the same genotype (a test of responsiveness to environmental influences); (2) by the proportion of previously known factors for other characters which are able to influence the character under consideration (a test of responsiveness to genetic influences). To both of these tests truncate gives a positive result, for we have seen, (1) that, when flies carry T_2', the wing shape is unusually variable even among individuals of identical factor composition, and (2) that, among these flies with T_2', a number of factors, like that for black body color, star eyes, bar eyes, sex, etc., which ordinarily produce no noticeable effect upon the wing, now influence its shape decidedly. Hence the developmental reactions that determine the wing shape must be particularly unstable, or susceptible to modification, in the race containing T_2'. From this conclusion in turn it follows that relatively many modifiers of wing shape should be found to arise by mutation in the truncate race,—not because mutation itself happens oftener in this race, but because, of the mutations that do occur, more would be able to affect the wing character in this race than in non-truncate races, where wing shape was not so easily influenced. A race of this kind, accordingly, would be able to undergo an evolution or cumulative change, under the directive action of selection, which would be next to impossible in another race, that lacked the factor causing this embryological modifiability. Here again, then, we have an example of invisible peculiarities of the germ plasm of certain lines, influencing the possibilities of character-variation and evolution.

The question might here arise—if characters may differ so considerably in regard to the modifiability of their development reactions, is it not conceivable that certain characters may be so highly modifiable,—influenced by such a large proportion of the total number of mutations that occur,—and that this *total* number of mutations is at the same time so large, that, even though each separate single factor of the germ plasm is highly constant, nevertheless these characters would, in effect, really be undergoing a continual genetic fluctuation. This contingency cannot be categorically denied, as an academic possibility for exceptional cases, but we have seen that in the case of truncate, although it is a particularly unstable character, yet the number of mutations which affected it were not nearly so frequent as would be required for such an effect,—the pure line remaining constant through twelve generations. Of course, if selection had continued indefinitely, some distinct genetic changes affecting the character would have been bound to occur, but not of the order of frequency of "continuous variation" (fluctuation). The reason that the inbred stock, on the other hand, was so much more plastic genetically is simply because there was in it an opportunity for the formation of all sorts of genetic types by mere recombination of the various mutant factors that had already arisen at widely separated occasions. The inheritance of truncate therefore provides no evidence even for this modified type of genetic fluctuability which might appear as the resultant of innumerable mutations in many different factors, each of which itself was highly constant. Moreover, no other cases affording evidence of such a situation have been reported thus far.

We may now sum up the causes why the truncate character, when it was analyzed, was found to depend on so many factors. (1) Two successive mutations were practically necessary, in the first place, before truncate became visible at all. (2) Since truncate depended on a lethal, and was, in addition, inconstant in somatic expression, it was subjected to selection, which perpetuated any new factors (balancing lethals and intensifiers) that still further differentiated truncate from normal. (3) Since the truncate character depended on an unstable developmental reaction, the initial appearance of such intensifiers was made more likely.

The history of the truncate stock, after the appearance of these contributing factors, consisted merely of the attempt to get the line as pure as possible for T_3', and to keep the line in the balanced lethal condition by eliminating crossovers.

THE ORIGINATION OF TRUNCATE IN OTHER STOCKS

It is not very uncommon to find truncate appearing sporadically, like a mutant, in other stocks. Thus, in DUNCAN'S (1915) search for mutations, truncate was the second most common in occurrence, beaded arising most frequently. Now, if the above account of the origin of the truncate complex has been correct, such truncate should not be provided with the accessory factors present in the selected stock, or at least not with the same ones. That this is true is indicated by the fact that often, when these truncates are bred, they fail to reappear, except in trifling proportions, in subsequent generations. The latter flies, then, if they owe their truncation to the same factor as the T_2' of truncate stock, evidently lack all the intensifiers, just as we should expect to be usual in stock not subjected to selection. In this connection, it may be noted that since the intensifiers are missing it is by no means certain that all these appearances of truncate represent separate mutations, as the factor for truncate may have been present for a long time, under such circumstances, without manifesting itself. Hence the actual mutations may really be rather rare.

Truncate has also appeared several times in stock containing factors for other characters, that at the same time intensify truncate, and these cases may with more certainty be regarded as real mutations. The composition of such truncates is accordingly more worthy of examination. One such truncate was found by MORGAN in a stock with "cut" wings; special crosses proved that it contained T_2', identical with the T_2' of truncate stock. Cut, itself, intensified the truncate, but there was no evidence of the regular intensifiers in addition. Another truncate was found by the writers in a cross between black and a stock that had been continually tested by crossing to bar. The mutant, a "long-truncate" female, was crossed to black pink, and gave offspring half of which were intermediate and the rest normal-winged. Two of the heterozygous intermediate F_1 males were then back-crossed separately to black pink. As in the similar crosses of ordinary truncate, truncate appeared only among the gray offspring (F_2), but it appeared about as often among the gray pinks as among the gray reds, giving in both classes the percentage and grade ordinarily present among the gray pinks. Thus it was evident that the intensifier T_3' was not present. The T_2' was evidently the same as that in ordinary truncate stock, for when the gray pinks $(\frac{T_2'B}{b}\,\frac{p}{p})$ were inbred they produced 81 grays and 51 blacks, instead of a ratio of 3 : 1, thus showing that this T_2' also was lethal. There was, moreover, a fairly large percentage of crossing over between black and truncate.

We may accordingly conclude that the origin, by mutation, of the intensifiers present in truncate stock, was quite independent of the origin of T_2' itself by mutation.

APPLICATIONS OF THE METHODS

It is believed by the authors that the general method of attack developed in the truncate case,—whereby, by the use of "identifying" genes, a refractory character may be taken apart, put together, or held in a desired combination—will become of more widespread applicability as the linkage groups of the organisms commonly used for genetic study become better known. Since a large part of the work on truncate was carried out nearly seven years ago this general method has already had a chance to be tried out rather extensively in Drosophila work, and it has been used with success by students of this organism in a considerable number of problems, some of which have been reported in the literature.

In the case of other organisms, however, it will be necessary to know and have the use of identifying factors in the various linkage groups concerned, before the present method can be used at all. Moreover, certain modifications are necessary in the case of organisms that have crossing over in both sexes, and also in case the character to be studied is recessive. In either of these circumstances, it will be desirable to have two identifying factors in each chromosome involved, one on each side of the factor under investigation,—in order to make sure that the factor has not crossed over from its identifier.

Remote as the possibilities of such work may seem to be in the case of such animals as mammals, it is nevertheless difficult to conceive how the genetic bases of the more elusive and complicated characters in them can be determined adequately by any other means. Even in the case of man, an attempt in such a direction would be justified, for here the most important characters,—such as the psychological ones,—are perhaps more plastic, obscure, and complicated in genetic basis, than any others in the entire animal kingdom, and it would seem next to impossible ever to give any real Mendelian analysis of most of them without studying them by the method of linked identifying factors. In place of controlled crosses, and large families, however, reliance would here have to be placed on finding, by means of wide examination of data, a sufficient number of crosses of similar type, and then seeking in them the requisite identifying factors.

It would accordingly be desirable, in the case of man, to make an extensive and thorough-going search for as many factors as possible that could be used in this way, as identifiers. They should, preferably, involve character differences that are (1) *of common occurrence,* that are (2) *identifiable with certainty,* and that are (3) *heritable in a simple Mendelian fashion.* It seems reasonable to suppose that in a species so heterozygous there must really be innumerable such factors present, if only an examination of the inheritance of *small, definite physical traits** were made on a large scale. As the study of such factors should naturally be accompanied by an examination of their inheritance with relation to each other, a knowledge of their grouping would at the same time gradually become available. All this would of course require very detailed and intensive work (rather, perhaps, than a superficial study of numerous individuals), and as yet little work has been done that is of this character. For, hitherto, the study of factors which are inconspicuous, or unimportant in actual life, has been largely avoided, in order to make an immediate and direct attack on the more important, more difficult characters. Now, as any two parents of a human family would probably differ nearly always in a very large number of factors, it would not be at all surprising if it were found that there were usually differences in one or more identifying factors in the case of any given one of the twenty-four (±) pairs of chromosomes. Thus, if a far-reaching investigation of definite physical traits were carried out first, then, when investigation of the more difficult characters was later undertaken, the requisite identifying factors, suitably arranged in the appropriate chromosomes, would probably be found, in many crosses, ready provided for the study of the more complicated and important trait.

The implication is not intended that no results of importance in human genetics can be attained by simpler methods; it would of course be desirable to carry on such studies at the same time as the more rigorous ones, but the inconclusiveness of the pedigrees of most important human characters, when studied directly, and their resemblance to the early pedigrees in the truncate case, indicates that nothing like an adequate understanding of the intricacies of inheritance in man can be reached without some such far-reaching and difficult plan as that just outlined. Meanwhile, too, partial knowledge of "identifiers" would be of partial help, in the larger problems.

However the situation may be for human genetics, it does seem clear that in the more tractable organisms, such as the domesticated and laboratory races of animals and plants, character analysis by means of linkage studies with identifying factors will come into more general use,

* Or chemical, such as the blood agglutinins.

both for the investigation of general problems and for the dissection of particular characters.

RESUMÉ

The general hereditary behavior of truncate

1. Truncate is a variable character, appearing in all grades between short-truncate and normal wing. Some normals are almost always thrown, even by the "best" truncates, and it was found impossible, through four years of selection, to secure a permanent stock that threw no normals, although the proportion of the normals was reduced to about 10 percent, and the average grade of the truncates which did appear was increased markedly at the same time.

2. This variation is not only somatic, but also genetic, for in the selected truncate race the high-grade truncates that appear throw relatively more and "better" truncates than do the intermediates, and the latter in turn greatly surpass the normals in this respect. It is thus possible, in the final stock, to modify the average grade and percentage of truncates back and forth by selection, within the limits above stated.

3. When truncate is crossed to the wild-type fly it behaves as an incomplete recessive, the great majority of F_1 being normal, but a small percentage of flies that show some truncation usually appearing also. The ratios in F_2 vary greatly with individual pairs of F_1 flies, ranging from nearly four normals to one with truncation through various values down to over 100 normals to one truncate, while some of the F_2 families contain no truncates at all. The percentage of truncates in F_2 is higher if the P_1 truncate was a female, but in either case the truncates in F_2 consist of both males and females in comparable numbers. The extracted F_2 truncates throw about two normals to one truncate but can often be improved by selection until they reach the limit shown by the selected stock.

4. It was found that on crossing to flies with black body-color (recessive), truncate behaves as a dominant, and manifests itself in some degree in nearly all of the F_1 flies that carry it. Two heterozygous factors, ordinarily recessive, may thus reenforce each other so that one becomes a dominant. Similar results were obtained in crosses with bar-eyed flies, and with star-eyed flies, but bar and star are both dominants themselves.

The dissociation of the truncate genotype

1. Truncate was crossed to stock having black body-color (chromosome II) and pink eye-color (chromosome III). In this cross, truncate could be used as a dominant (since black is present), and F_1 truncate males were back-crossed, separately, to black pink. In this way flies having all possible combinations of the chromosomes present in the truncate stock were obtained, and could be recognized, according to their sex, body color, and eye color, these latter being used as "identifying factors" for the chromosomes containing them. By then studying the amount of truncation in the flies having the different combinations, the effect of each of the chromosomes of the truncate stock, on the truncate character, could be determined. The following results were arrived at:

(a) There is a chief factor for truncate (T_2'), lying in the second chromosome; flies having only T_2', in heterozygous condition, and no other mutant factor for truncate, may occasionally have truncated wings. T_2' is partially dominant.

(b) The third chromosome of truncate stock also contains a mutant factor or factors for truncate, which may be designated as T_3'. This acts as an intensifier of T_2', and when it is heterozygous at least it produces no visible effect on the wing unless T_2' is present.

(c) These crosses, when made in both reciprocal forms, showed that there is a similar intensifier, T_1', in the first (X) chromosome, which intensifies truncate to about the same extent as does T_3'. When both intensifiers T_1' and T_3' are present they have a summative action. T_1', like T_3', produces no visible effect unless T_2' is present. T_1' is partially dominant, under the conditions of these experiments (with black present).

2. Crosses of truncate flies lacking T_1', obtained in these experiments, showed that the truncate character is also influenced by sex ("sex-limited"), in that it manifests itself more readily in females than in males.

3. Flies from these experiments, that were known to contain T_1' and T_3', but lacked T_2', were crossed with each other. As no wing modifications were produced, it was evident that both T_1' and T_3' together, in the absence of T_2', cannot cause truncation, or any visible effect on the wing, even when opportunity is given for them to become homozygous.

4. It was found possible to resynthesize truncate, according to calculation, from the flies of the non-truncate or imperfectly truncated classes of the dissociation experiment. Flies were chosen for mating together

which were scheduled, by their identifying factors, each to contain factors for truncate that were missing in the other. High-grade truncate then appeared in the offspring in the classes expected to contain it. This result furnished a confirmation of the conclusions from the dissociation experiment, and showed that the flies of the non-truncate classes had received the factors for truncate which they were supposed to have, and transmitted them regularly to their descendants.

5. In the crosses just mentioned, T_3' was sometimes introduced through the mother, sometimes through the father. The slightly lower grade of truncation, when T_3' is derived from the mother, indicates that T_3' is multiple in composition, consisting of two or more factors that may cross over from one another in the female.

6. Similar tests with T_2' indicated that this includes only one factor (at any rate one dominant factor).

7. It was found in later crosses that this "chief factor," T_2', is located in the "left-hand" end of the second chromosome, about 12 units to the right of star.

8. Experiments involving, in addition to black and pink, a fourth-chromosome factor, bent, showed that the truncate stock does not contain an intensifier in chromosome IV. In these latter experiments the distribution of the entire germ plasm was under observation.

Tests of the constancy of the factors for truncate

1. Individual tests were made of brother and of sister flies from the F_2 count of the "dissociation experiment," which could be seen, by their identifying factors, to have received from their father a given chromosome combination,—T_1' T_2' T_3' in the case of the sisters, T_2' T_3' in the case of the brothers. The tests showed that the differences in truncation between the flies having a given combination were not genetic; that is, the flies which receive the same chromosome combinations from their parents contain identical factors for truncate. It must be concluded from this that the factors for truncate follow exactly the distribution of the chromosomes, and that they do not undergo fluctuating variation.

2. A "pure-line" experiment was then undertaken, by repeated back-crossing of males of the above sort $(\frac{T_2'B}{b}\ \frac{T_3'P}{p})$ to black pink females. In each generation, in such a cross, males with the same truncate combination as their father are again produced, and may be recognized by their identifying characters (gray body and red eye-color). These are

then mated, as before, to black pink females from stock, and so the process may be repeated indefinitely. The danger of recombination is here excluded as effectually as in the case of self-fertilizing or asexually reproducing organisms, and so the purpose of a pure-line experiment is fulfilled. Two lines of flies were carried on in this way,—a "high" or plus-selected line, for twelve generations, and a "low" or minus line, for thirteen generations. The selection was absolutely ineffective; this corroborates the conclusion derived from tests of brothers and sisters, and shows that the factors for truncate are constant, and entirely contained in the (three large) chromosomes. It proves at the same time that the factors for truncate are not contaminated by their allelomorphs in the heterozygote.

The continual genetic variation occurring in the truncate stock must therefore be due to recombinations of factors for which the stock is perpetually heterozygous.

The cause of the inconstancy of the selected stock

1. To determine the reason for this perpetual heterozygosis of the stock, tests were undertaken to discover whether T_2' could exist homozygously. As it was next to impossible to determine this by direct tests, T_2' was placed in a chromosome with *b* (black), and flies with this combination were crossed to others having T_2' bound to *B* (gray). 72 offspring which had received at least one T_2' were then tested, by backcrossing to black, to discover whether they contained both the T_2' with black and the T_2' with gray. None of them, however, had more than one of the T_2' factors, and the ratios showed that T_2' acts as a lethal when homozygous.

Thus T_2' resembles the majority of dominant mutant factors of Drosophila in being lethal when homozygous, and pure stock of it cannot be obtained.

2. It follows that crosses of truncates should yield 1 non-viable: 2 potential truncates : 1 normal. As in the selected stock much fewer than one third of the flies that hatched were normal, tests were made to see whether any of the normals that should have occurred had been caused to appear truncate by reason of some other, recessive, "chief factor" for truncate. It was found, however, that no such factor existed; consequently the deficiency of normals was due, not to their appearing truncate, but to their actual absence,—this means that they were prevented from hatching by a lethal factor, which must have lain in the chromo-

some homologous to that bearing T_2'. Occasional crossovers between T_2' and the lethal would give rise to a few normals, however.

It is thus evident that there is in truncate stock a condition of "balanced lethal factors," similar to that existing in beaded Drosophila, Matthiola, and Oenothera.

3. Reciprocal out-crosses of truncate stock showed that T_1' was present, homozygous, in viable, fertile flies of the selected stock.

4. Crosses of flies from the dissociation experiment were arranged in such a way that T_3' was given opportunity to become homozygous, while T_1' and T_2' remained heterozygous. Many of the flies thus synthesized were as high-grade truncate as the best inbred stock, and much higher than the truncates from out-crosses. This proved that T_3', when homozygous, is not lethal, but that its effect on truncation is much stronger than when heterozygous. Females of this composition are so infertile, however, that it would be next to impossible to maintain stock pure for T_3'. This explains the occurrence of low-grade truncates, and normals carrying truncate, in the selected stock.

The origin of the truncate complex

1. Truncate is one of the most frequent characters to appear sporadically, by apparent mutation, in various stocks of Drosophila. In most of these cases, however, intensifiers of truncate are absent, and so the possibility is not excluded that truncate was present previously in the stocks, and merely failed to manifest itself before. In accordance with these circumstances it is usually found that the truncate fails to reappear in the descendants of such flies, except in a minute percentage of individuals.

When, however, truncate appears in stocks containing bar, or other factors which themselves intensify truncate, it may be concluded that truncate has here arisen as a real mutation. Several such truncates have been tested, and found to be inherited, and evidence was obtained that their mutant factor for truncate was identical with T_2', the chief factor of ordinary truncate stock. None of the intensifiers of the ordinary stock were present, however.

2. The hereditary behavior of the original truncate shows that one of the factors for truncate had been present, undetected, in the parental long-winged stock, and that the appearance of truncate was due to the origin, by mutation, of a second factor. Thus the first truncate-winged fly contained T_2' and an intensifier. The other mutant factors of the

truncate stock appeared later, and they were perpetuated through the process of selection, because they influenced the truncation in the direction desired.

It is of significance for evolution, as well as for genetics, that particular races may thus have pre-existing in them factors which by themselves are invisible, but which favor the manifestation of a certain character.

3. The occurrence of more modifiers for truncate than for most characters is explained not only by the fact that the modifiers were specially picked out by selection in the case of truncate, but also by the marked instability of the developmental reaction whereby the truncate character is produced. This instability, or susceptibility of modification, is disclosed by (1) the somatic variability of the character, when the genotype is kept constant, and (2) by the number of mutant factors for other characters which act upon truncate in addition. On account of this modifiability it should happen that relatively many of the mutant genes which arise would be able to affect truncate, and it should therefore be easier to find either intensifiers or "inhibitors" for truncate than for most characters. The reason for the readier discovery of modifiers would hence be, not that the process of mutation is influenced in some way by truncate, but, on the contrary, that truncate is especially susceptible of being influenced by mutation.

The existence, in certain races, of factors like T_2', which make a certain character (here, the wing shape) more highly modifiable, may be of particular importance in evolution.

General applications

1. The results described in the first section of this resumé would undoubtedly have been regarded by certain writers as positive proof of the ineradicable fluctuability, miscibility, or, as it were, fluidity, of a unit character. The case was, in fact, more extreme than those upon which they rely as evidence for their doctrine. And yet it has been found that the inheritance of this character is entirely confined to the recognized chromosome system, and depends on definite chromosomal factors which are neither miscible nor inconstant.

Although mutations affecting this character may occur with somewhat greater frequency than those for many other characters, the mutations are not nearly of the order of frequency which would be necessary in order for them, by themselves, to give rise directly to a true genetic fluctuability. Practically all the genetic variability and modifiability of the stock is, on the contrary, due to the occurrence of recombinations among those mutant factors which have, on rare occasions, previously arisen, and variation due to a real new mutation, although possible, is highly exceptional. Even in such cases, moreover, the mutations do not all consist in variations of some one particular factor, but they may affect any one of many factors that are concerned with the character.

The use of cases resembling the truncate case, in order to support the doctrine of fluctuating variability of single unit-factors, is therefore entirely unjustifiable, in the absence of analyses comparable with those made in this case.

2. It is believed that the general methods developed in this case will become increasingly useful with the growth of knowledge of the linkage groups in organisms. It has been shown how, by the use of "identifying factors," any given genotype may be cut up for study, put together again in various ways, or held in a particular combination for the maintenance of stock or for observations on factor constancy. At the same time the distribution among the offspring, of the entire (chromosomal) germ plasm of a parent may be completely exposed to view.

The suggestion is made that such methods may also be necessary before much progress can be made in the study of the more important characters in human genetics.

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SPOTTING INHERITANCE IN *DROSOPHILA BUSCKII* COQ.[1]

DON C. WARREN

Georgia State Board of Entomology, Valdosta, Georgia

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[1] From the Zoölogical Laboratory of INDIANA UNIVERSITY. Contribution No. 171.

INTRODUCTION

Few groups of animals or plants have received so much study by the geneticist as the fruit fly of the genus Drosophila. This work, carried on largely by MORGAN and his co-workers, has consisted almost entirely of an analysis of the inter-relations of variations found in the laboratory. Large numbers of flies have been minutely examined for germinal variations and this critical study has resulted in the finding of a large number of mutations of various sorts. These mutations were usually observed soon after their appearance and further work with them has resulted in adding much new and valuable light to our knowledge of heredity.

Although many mutations have been found in the laboratory, very few have been recorded in flies taken from nature. This fact has led some writers to question whether these variations found in the laboratory are really potent factors in the evolutionary process. The difference may lie in the fact that flies in nature have not been given the exhaustive study which has revealed the mutations appearing in the laboratory. However, when one examines any large number of flies in nature he is struck, not by the large number of variations which may be of evolutionary value, but rather by the lack of these. There is certainly very little evidence of any variations similar to those found in the laboratory, which seem to be taking the place of any existing contrasted characters.

THE PROBLEM

The problem of this paper has been to analyze variations which were found in nature. In studies of this sort it is not possible to know anything concerning the origin of the variations and hence the analysis is more difficult. The variation studied was the spotting of the abdomen. The study of the variations in the male, together with a variation in female spotting, which later appeared in the writer's stocks, composes the work of this paper. In its final form the problem became an attempt to analyze critically the factors which govern or influence the spotting character or characters in the fly. To narrow the field still further the writer has limited himself to the spots on a single segment of the abdomen. Whether the inheritance of the spots on the other segments of the abdomen are dependent upon the same factors remains in question.

DESCRIPTION OF THE SPECIES

The fruit fly *Drosophila buschii* is slightly smaller than the well known

Drosophila melanogaster and is readily distinguished from it by conspicuous spots on the abdominal segments. Each segment, excluding the last two, bears six spots. These spots are arranged on each segment as follows. There is a pair of rather large distinct spots on the dorsal surface of the segment, one on each side of a mid-dorsal line. A second pair appears more laterally, one on each side just below the last-mentioned pair. The third pair of spots are small ones, latero-ventral in position. The spots are arranged so as to make six rows running lengthwise of the abdomen. There is seldom any variation in the spotting of the above-described segments.

The spotting of the fifth segment differs from these, however, and the variations found on that segment furnish material for this study. In the female it is like that of the other more anterior abdominal segments,[2] but in the male there are variations. The large dorsal pair of spots is always present and the presence of this pair alone seems to be the more common type of spotting among males found in nature. Either one or both of the other two pairs may be present or absent or any one spot of a pair may be combined with any other spots, thus giving all the possible combinations of the constant dorsal pair and the other four variable spots. These various combinations are shown in figure 1. The spots show a tendency to be present or absent in pairs, that is, if the outside spot on one side is missing the corresponding spot on the other side is also likely to be missing. This tendency is especially true of the outside pair of spots and one outside spot occurs without the other so infrequently that no record of this type of spotting has been made in the tables which follow.

MATERIAL AND METHODS OF BREEDING

The flies used in these experiments were collected in the vicinity of Bloomington, Indiana, during the autumns of 1915 and 1916. In nature they breed in decaying fruit and vegetable matter but most frequently in rotting tomatoes. At first it was attempted to breed them on banana in the laboratory. It was found that they could be reared on this food, but with some difficulty. Later, moistened bran was tried and used very successfully, being a food which is both convenient to handle and one upon which the *Drosophila busckii* breeds very well. This species is very prolific and requires about fourteen to twenty-five days for a generation, depending upon the temperature. It does not well withstand high temperatures and the extreme summer heat encountered in central Indiana sometimes killed stocks of flies.

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[2] One exception was found which will be discussed later in the paper.

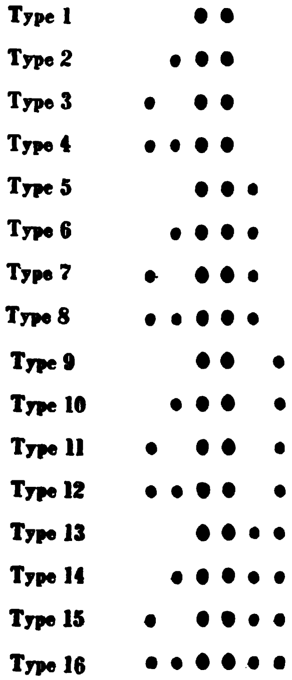


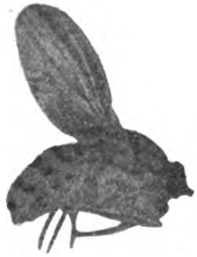
FIGURE 1.—Diagram of the various types of spotting found on the fifth abdominal segment of the male. Each type represents a flattened segment showing the spots upon it. The large dorsal pair was always present and was found in every possible combination with the other four smaller spots. The spots tended to be present or absent in pairs and are referred to throughout the paper as the dorsal pair, the middle pair and outside pair. Type 1 was most common in nature.

MALE SPOTTING

Selection lines

This study was suggested by the observation that there were variations in the spotting of the males. Preliminary work upon these variations indicated that they were heritable to a certain degree. The earlier work consisted largely of crosses between the more extreme variations to see how they would behave, genetically. Further than establishing to the writer's satisfaction that these variations were heritable, very little was accomplished, for no pure strains of any of the variations had been acquired. So it was decided that the best method of procedure would be to try to establish by selection, strains which were pure for some of these variations. Selection was made for the two extremes of the various types of spotting in the males. These two extremes were the normal or two-spotted condition (fig. 1, type 1, and plate 1, no. 1) and the six-spotted condition (see fig. 1, type 16, and plate 1, no. 3). As well as

WARREN, D. C., SPOTTING INHERITANCE IN DROSOPHILA BUSCKII PLATE I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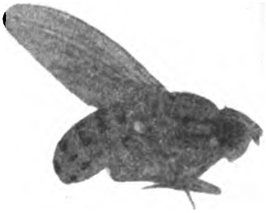
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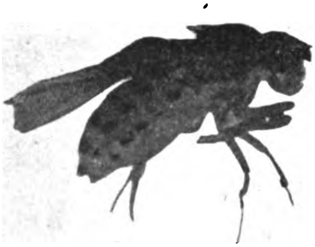
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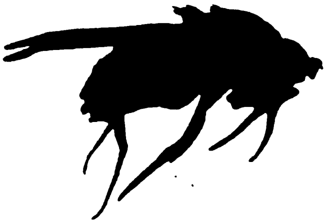
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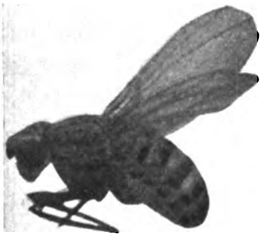
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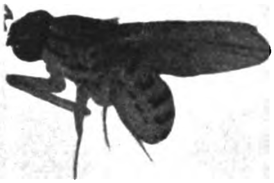
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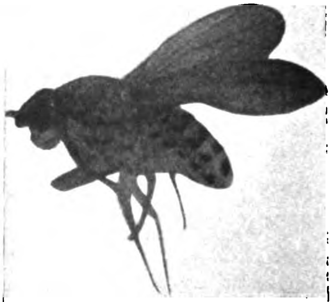
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GENETICS 5: 64 Ja 1920

LEGEND FOR PLATE I

Some of the types of spotting found in *Drosophila busckii.* The work of this paper is confined to the fifth abdominal segment (the last segment showing spots).

No. 1.—Normal male with only the dorsal spots.
No. 2.—Male with dorsal spots and small outside one.
No. 3.—Male having full set of three pairs of spots.
No. 4.—Normal female with full set of three pairs of spots.
No. 5.—Abnormal female with middle pair of spots missing.
No. 6.—501 type of male with very large outside spots and also small middle spot.
No. 7.—Male with dorsal spots and small middle spot.
No. 8.—501 type of male with large outside spot.

being an attempt to isolate two desired types of males the problem was outlined as a selection problem. From flies taken in nature three stocks were started which were not known to be related. To begin with quite a number were examined in each stock to learn what was the ratio of the various types of male spotting in each. Then from among the offspring of a single pair in each stock a "high" and a "low" strain were started. The high strain was one in which the paternal parent in each succeeding generation was to be a male with a full set of six spots, while the low strain was one in which the father of each generation was to be a male of the normal or two-spotted type. At the same time that these two strains were started a control strain was also started in each stock. In the control the parents were selected at random with no knowledge of the nature of the spotting of the fathers. The three stocks in which the selection was started were known as 500, 502 and 504 and after the selection was started there was a low strain, a high strain and a control strain in each.

Selection line 500

Three pairs of flies were taken in nature and placed in a bottle which was numbered 500. This was the origin of the strain 500. A record was made of the spotting of the offspring from this bottle and six pairs of the offspring were mated *inter se.*[8] Of these six matings, one was used as the starting point of the high and low strains of line 500. This mating was a single-pair mating and the female was virgin, so all of the offspring were necessarily full brothers and sister. This gave the two strains as nearly uniform starting conditions as possible. The only difference in conditions at the starting of the high and low strains was that two-spotted males were used in the low strain and six-spotted males were used in the high strain. In as far as possible uniformity of all conditions except the one mentioned was maintained during succeeding generations of selection. Tables 1, 2, and 3 show the selection in the three strains of line 500.

In this line a control was not started at the beginning of selection but was originated by mating the high and low strains at generation 3. All succeeding generations of the control were mated at random with no knowledge of the type of male used.

[8] It will be noted that the counts obtained from the earlier matings were rather small as compared with the later generations of selection. This was due to the fact that the flies were at first reared on banana upon which they do not thrive well. Later wet bran was used and found much more satisfactory.

Table I
Selection in strain 500 low.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	56	152	14	11	7	7	22		9	3	48	273
1	28	91	9	13	5	3	145	7	6	11	36	326
2	85	430	4		2		66		2		1	505
3	92	1166	6	11	12	1	46	1	8		17	1268
4	96	606	7	6	6	1	8	1	2		5	642
5	99	717	4		4							725
6	94	239	5		5	2		1	1		1	254
7	97	477	3		10	1			1		1	493
8	97	433	3		10							446
9	99	347	2		1							350
10	94	546	5	2	6	8	9				3	579
11	93	435	7		5	2						469
12	99	372	1		1							374
13	99	350	1		1		1				1	354
15	89	181	1		1	1	17	1			2	204

Table 1 shows the selection for the two-spotted type of male (type 1) in strain 500 low. The column showing the percent of selected type gives a fair idea of what was accomplished by selection. The column headings, type 1, type 2, etc., refer to the types of male spotting found. These types are shown in figure 1. Some of the types given in figure 1 are omitted in the tables which follow. This is due to the fact that there were so few individuals in some of the types that space was not given them in the tables.

In generation one of the low strain eight matings were made but in practically all of the succeeding generations two stock matings were made in each generation of each strain. The graph shown in figure 2 gives a fair idea of the results obtained by selection in this line. Selection was much more effective in the direction of the two-spotted males. The graph representing the low strain in generation 4 drops to near one-hundred percent two-spotted males and remains so during the rest of the selection. Selection in the opposite direction was less progressive. The graph representing the high strain is very irregular and a high percentage of six-spotted males was not reached till generation 9. During the first eight generations of selection the high strain frequently threw more two-spotted males than six-spotted ones. This seemed to be characteristic of line 500. There was a tendency in all strains to produce a high number of two-spotted males and the percentage of six-spotted was difficult to increase. The control in which there was no selection, al-

TABLE 2

Selection in strain 500 high.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	18	152	14	11	7	7	22		9	3	48	273
1	21	10		1			17	1		2	8	39
2	43	29	1	4	3	1	22	4	2	2	50	118
3	24	179	8	24	7	4	47	4	24	7	98	402
4	43	122	6	27	7	4	51	5	22	6	186	436
5	7	348	19	15	25	22			17		35	481
6	12	360	20	22	26	22	5	4	12	3	62	536
7	17	335	19	28	20	18	27	4	26	6	100	583
8	34	146	2	18	14	7	90	19	18	15	171	500
9	79	1		2		1	13	10	2	8	138	175
10	61	99	10	12	23	28	49	23	6	24	422	696
11	56	22	1	3	2		134	21	2	18	261	464
12	86	9	3	2	6	4	13	4		2	268	311
14	66	8					42	5		3	111	169

Table 2 shows the selection for the six-spotted type of male (type 16) in strain 500 high. The types referred to in the column headings are found in figure 1. The increase in percent observed as one reads down the second column shows what was accomplished by selection.

TABLE 3

Counts for control strain of line 500.

Generation	Types of male spotting										Total males
	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	152	14	11	7	7	22		9	3	48	273
1	195	6	1	6		11	2	1	1	11	234
2	134	1		1	1						137
3	184	5	5	12	2		1	2		6	217
4	209	6	2	5	3			1			226
5	199	8	8	4	8	45	3	3	6	23	307
6	179	2		2	1						184
7	123	1		4	1						129
8	109	3	1	4	3	9				10	139
9	148	13	2	10	6	6	1		1	1	188

Table 3 shows the counts for each generation of the unselected strain 500 control. These counts should be compared with those in tables 7 and 2. The behavior of this strain in relation to the two selected strains in line 500 is best shown in figure 2.

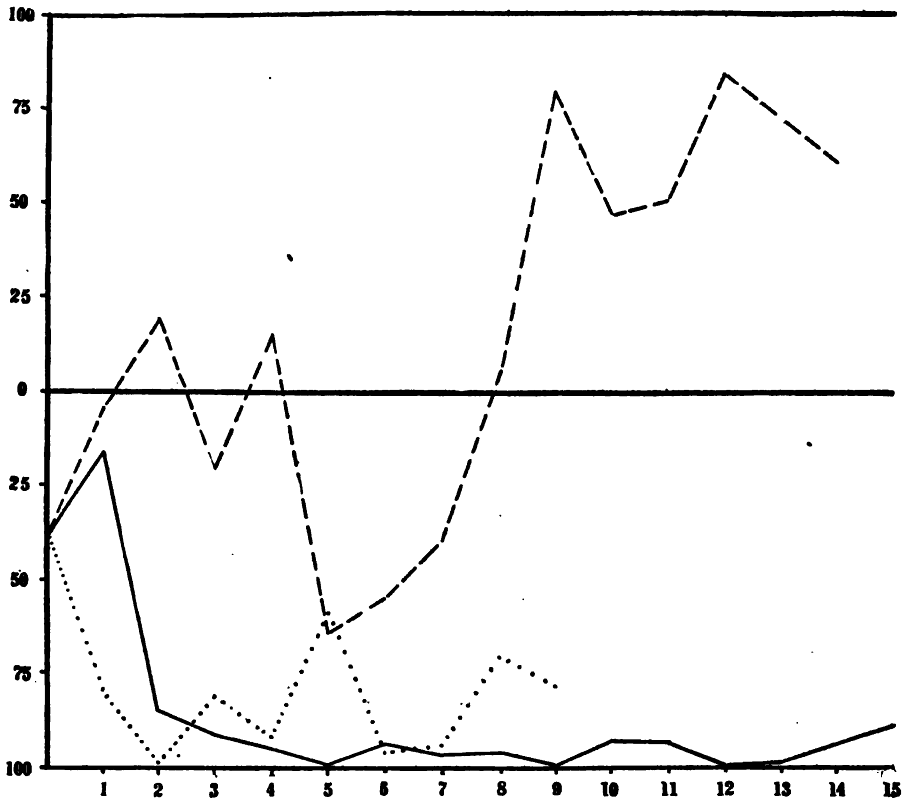

FIGURE 2.—Results of selection in line 500. The dash line represents the high strain, the dotted line the control, the solid line the low strain. The abscissae represent the generations of selection while the ordinates represent the difference between the percentage of two-spotted and six-spotted males in any generation. Differences in favor of the six-spotted males are plotted above the zero line while those in favor of the two-spotted males are plotted below the line; *e.g.*, in generation 2 of the high strain, 43 percent of the males were six-spotted and 24 percent were two-spotted, so the difference was 19 in favor of the six-spotted males and was plotted at 19 above the zero line. The data for this graph are found in tables 1, 2 and 3.

though somewhat more irregular, did not range far from the low strain. The extra spots of the six-spotted males of the high strain were all rather indistinct as compared with those in line 502.

Results

There can be no doubt but that the high and low strains have been made different by selection. Although a much lower percentage of males of the desired type were obtained in the high strain than in the low strain, it is evident that more was really accomplished by selection in the high strain. This is true because the line 500 at the beginning had a tendency to produce two-spotted males and by selection a greater difference was brought about between the high strain and the control than between the control and the low strain.

Selection line 502

This selection experiment (tables 4, 5 and 6) was started and carried out in the same manner as on line 500. The initial stock was taken from nature, bred in the laboratory for two generations and then from among the offspring of a single pair the selection strains and control were started. This line soon showed itself to be characteristically different from line 500. Line 502 showed a tendency to produce a high percentage of six-spotted males. The graph (figure 3) representing the behavior of the low strain in this line was much more irregular than the

TABLE 4
Selection in strain 502 low.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	31	78	8	14	4	10	30	1	10		98	253
1	22	26		1	1		73	2		3	11	117
2	24	96		9	2	3	123	6	12	8	135	394
3	82	173	1	2	2		26		2		4	210
4	94	739	5	14	7	1	12		8		2	788
5	85	622	24	3	26	9	4	4	7		28	727
6	84	722	19	7	23	15	14	3	11	2	39	855
7	93	480	7	7	13	4	2		1	1	3	518
8	94	410	4	1	4	2		1			2	424
9	79	586	18	13	25	23	33	5	6	8	27	744
10	32	92		1	4	1	166	7	1	4	8	284
11	54	230	3	2	6	5	139	8	5	15	12	425
12	81	370	8	3	10	1	52	4		3	7	458
13	50	78	3	1		1	48	13		2	9	155
15	47	98	2		1		94	1		2	11	209

The counts in table 4 show the results of selection for the two-spotted type of male (type 1) in strain 502. The types referred to in the column headings are shown in figure 1. The second column shows the effect of selection and the decrease in the percent of selected type in the latter part of the experiment will be seen to be due to an increase in the type 11 males. The type 11 male (see figure 1) is one with only the outside spots and these spots are affected by temperature variations. Temperature is probably the cause of the decreased percent in the last few generations.

TABLE 5

Selection in strain 502 high.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	38	78	8	14	4	10	30	1	10		98	253
1	11	8					44	3		2	7	64
2	62	48	1	7	3		108	4	7	8	305	491
3	70	33		8	2	1	40	5	8	10	246	353
4	60	26	1	9			127	28	6	18	326	541
5	75	33	2	7	3	2	62	13	5	9	416	552
6	70	2	1	4			104	20	2	20	351	504
7	89			1			38	20	1	16	585	661
8	55			1			141	31		7	217	397
9	86						10	2		4	96	112
10	100									1	502	503
11	93						24	1			351	376
12	100						1	1			458	460
14	98						1			2	147	150

Table 5 gives the results of selection in strain 502 high. Selection was for the six-spotted or type-16 male. Figure 1 gives a diagram of the types referred to in this table. It will be seen from column two that it was possible by selection to obtain almost one hundred percent of the desired type.

TABLE 6

Counts for control strain of line 502.

Generation	Types of male spotting										Total males
	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	78	8	14	4	10	30	1	10		98	253
3	374	3	7	6	1	83	4	9	3	16	506
4	166	5	2	10	9	6	2		1	21	222
5	89	3			2			1		1	96
6	135	5	3	5	5	13	3	3	3	19	194
7	92	2	7	5	4	35	9	5	5	88	252
8	38		4	2	3	33	7	1	5	35	128
9	30	2	2	1		26		1	1	17	80
10	7		1			98	10		1	66	183
11	62		1	8	6	33	19		2	44	175

The counts in table 6 represent the behavior of strain 502 control. This was the unselected strain of line 502. The best comparison of the behavior of this strain with the high and low strains of 502 is shown in the graph in figure 3.

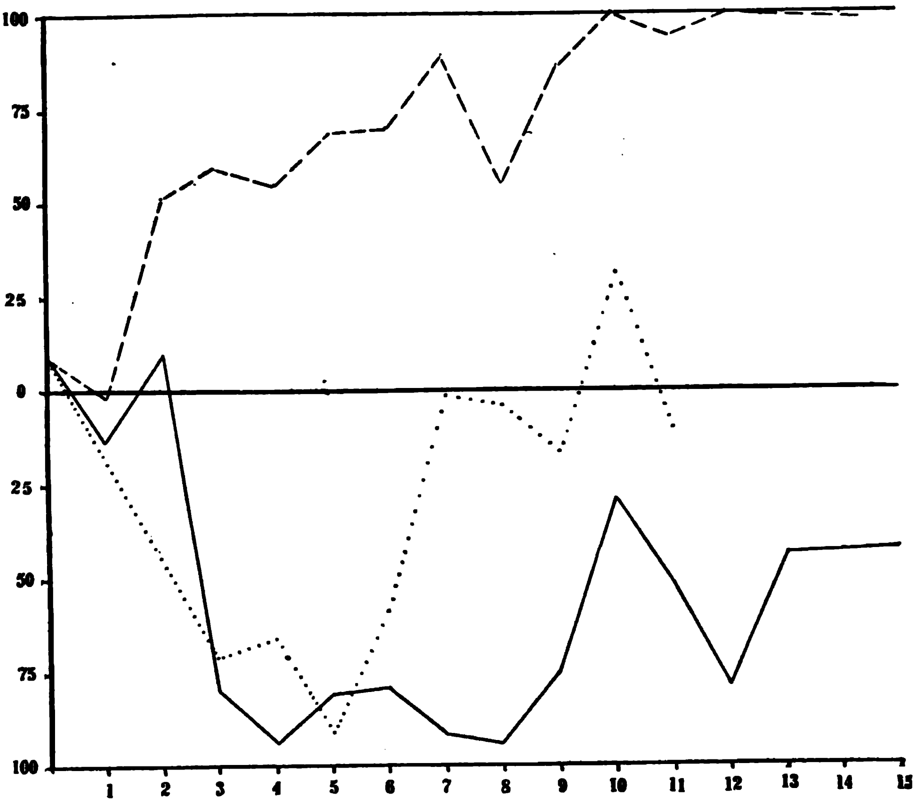

FIGURE 3.—Results of selection in line 502. See figure 2 for explanation of graph. The dash line represents the high strain, the dotted line the control and the solid line the low strain. Tables 4, 5 and 6 give the data for this graph.

corresponding one in line 500. Also the percentage of two-spotted males in the corresponding generations was lower than in strain 500 low. The percentage of six-spotted males in the high strain of 502 gradually increases and in the last few generations nearly approaches one hundred. The apparent loss of effect of selection in the last few generations in the low strain was probably due to temperature and will be discussed later. The extra spots on the males of the high strain of 502 were very distinct.

Results

Here again there is clear-cut evidence of results having been accomplished by selection. The graphs representing the two strains show a wide divergence and the control, although rather irregular, occupies an approximately midway position. There was a greater difference brought about by selection between both strains and the control than in line 500.

Selection line 504

The selection strains in this experiment were started as in the other two experiments. The data for this line are found in tables 7, 8 and 9. This line behaved somewhat similarly to line 500 and perhaps a little more extremely. There was a tendency to produce two-spotted males. Very early in selection (generation 2) the low strain produced nearly one-hundred percent two-spotted males as is shown by the graph and data for this strain. The graph (figure 4) then runs very uniformly near one-hundred percent except in the last two generations when there was a rather sudden rise. This irregularity was due to temperature and will be discussed under the topic of temperature. In the high strain the results were rather variable for the first few generations of selection. The percentage of six-spotted males was hard to increase and not until the last few generations of selection did the graph representing the behavior of the high strain rise above the zero line, thus showing a preponderance

TABLE 7
Selection in strain 504 low.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	31	117	15	14	7	14	89	7	12	1	97	373
1	93	332	1				25					358
2	98	1287	4	1	4		9	1	2		1	1309
3	99	632	1		1		1					635
4	100	235										235
5	98	437	2		2	4						445
6	99	547	5		2							554
7	99	458	2		1				1			462
8	98	452	4	1	4	1						462
9	92	462	3	1	3	1	29		1	1		501
10	97	302	2		5	1						310
11	99	389	1									390
12	98	406		1	3	1	3					414
14	62	133	1		1		78	2				215

Table 7 gives the selection for the two-spotted male (type 1) in strain 504 low. The types referred to in this table are shown in figure 1. It is seen that selection was very effective in this strain. All of the strains of line 504 showed a tendency to produce males of the two-spotted type. In this respect this strain is quite different from the low strain of line 502. By comparing the results of this table with those in table 4 this difference is readily seen.

TABLE 8

Selection in strain 504 high.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	26	117	15	14	7	14	89	7	12	1	97	373
1	2	106	4		2		81	2			4	199
2	3	384	6	3	6		45	2	5	1	14	466
3	4	728	27	13	42	33	67	3	16	3	34	966
4	3	521	17	5	16	12	50	4	6	3	21	655
5	10	429	14	13	17	15	193	23	8	13	80	805
6	6	429	18	19	24	26	117	21	8	9	46	717
7	6	219	12	6	20	12	89	8	7	3	22	398
8	14	238	19	14	17	15	170	19	5	20	82	599
9	4	39	2		1		199	6	2	13	10	272
10	32	2		1		1	221	25		18	127	395
11	51	62	8	6	19	14	86	16	5	8	230	454

In table 8 are shown the results of selection in strain 504 high. It was difficult by selection to increase the percent of six-spotted males (type 16) due to the tendency of all of the strains of this line to produce two-spotted males. However by examining the graph in figure 4 it will be seen that selection made the two selected strains act quite differently.

TABLE 9

Counts for control strain of line 504.

Generation	Types of male spotting										Total males
	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	117	15	14	7	14	89	7	12	1	97	373
1	290	7	1	15	1	33	1	3	1	8	360
2	131	1		4							136
3	138	3		1	2	8	1	1	1	2	157
4	193	5	1	7	5			1			212
5	242	8	4	7	3	18					282
6	105	1			1						107
8	147	7		7	1					5	167
9	114	2		7	1			1			125
11	110	1	1	4	2	11					129

Table 9 shows the counts for each generation of the unselected strain 504 control. The behavior of this strain in its relation to the two selected strains of line 504 is shown in figure 4.

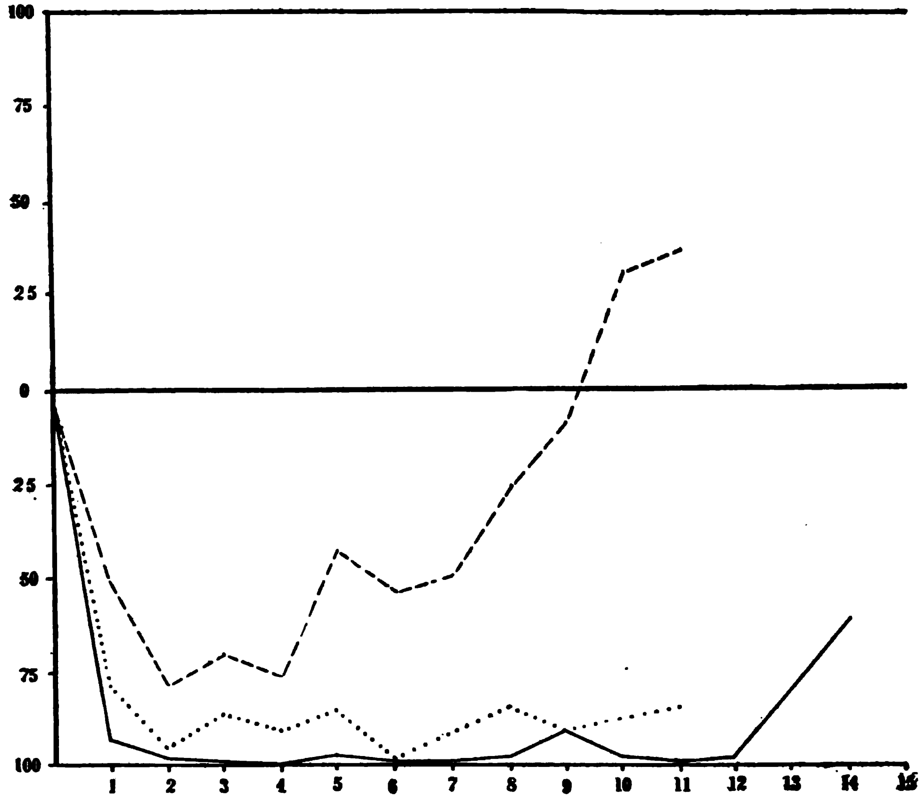

FIGURE 4.—Results of selection in line 504. See figure 2 for explanation of graph. The dash line represents the high strain, the dotted line the control, and the solid line the low strain. Tables 7, 8 and 9 give the data for this graph.

of six-spotted males. The percentage of six-spotted males never rose as high as in the high strains of lines 500 and 502. The extra spots on the six-spotted males in the high strain were much less distinct than in line 502. The control was not started from the original stock of line 504 but was originated as a cross between the high and low strains after two selections. The control threw a rather high percentage of two-spotted males but is shown by the graph as running more irregularly and slightly above the low strain.

Results

Although in line 504 it was impossible by selection to produce a very high percentage of six-spotted males, there can be no question about the significance of the results. The divergence of the two lines representing the high and low strains clearly shows what has been accomplished.

Discussion of the selection experiments

Selection has been carried out upon three distinct stocks of flies. In each case two strains have been produced which behave quite differently with respect to the character upon which selection acts. As to how selection acted is a more difficult problem. There are two conflicting views: first, the pure-line view that selection can only isolate existing pure lines, or the elimination and accumulation of multiple factors; second, the opposing view until recently held by CASTLE that selection modifies the gene.

This experiment offers no demonstration of the correctness of either view. In the experiment under consideration the object of selection was not to increase and decrease the intensity of a given character but rather to vary the percentage of individuals showing the character. The character was the presence or absence of four spots on a given abdominal segment. It was found that each line selected from nature seemed to have a more or less characteristic behavior with respect to the presence or absence of these spots. The ease with which a strain could be varied by selection in a given direction and the range of variation seemed to be a characteristic of the strain itself. The easiest explanation of this behavior is that this is a case of isolating pure lines. This in a way would be comparable to the classic experiments of JOHANNSEN upon beans. The difference in range of variation of the different strains is hardly sufficiently great to make a good case of this sort. However the rather striking difference in amenability to selection in a given direction does lend support to the pure-line theory and causes the writer to believe that the lines 500, 502, and 504 were all different genetically when taken from nature. It should be added, however, that at the end of the selection experiment the two high strains of lines 502 and 500 (see discussion of results of crosses between various male strains, which follow later in the paper) were crossed and the F_1 results indicated that at least some of the factors for spotting were common to both high strains. So although the two high strains reacted differently to selection and even remained somewhat different at the end of selection, they appeared to have the same genetic constitution at the end. Selection may have changed the genetic constitution of one of them. The writer is unable to see how the assumption of multiple factors would assist in explanation of this case and therefore merely presents the observed facts allowing the reader to give his own interpretation.

Permanency of the strains established by selection

An experiment was carried out to test whether the difference brought about by selection in the high and low strains was permanent. Line 502 was used in this test and at generation 5 of selection, a strain was isolated from each of the high and low strains. These strains were to be carried along with the selection strains but the male parents of each generation were to be taken at random with no knowledge of the type of spotting which they possessed. Random selection was carried on for seven generations and then counts were taken of the offspring. The results (table 10) show that there had been no retrogression in either strain in spite of the fact that selection had been stopped for several generations. In the high strain the count showed that seventy-six percent of the males were of the six-spotted type and at generation 5 when this strain was isolated to be carried without selection, the strain showed seventy-five percent of the males to be of the six-spotted type. So the high strain had not changed. At the time that selection was stopped, the low strain showed eighty-five percent of the males to be two-spotted and after seven generations of mass culture without selection it still showed eighty-nine percent of the males to be two-spotted. These two strains were carried for twenty-three generations without selection and the intention was to make another count after a long period. Pressure of other work prevented these counts from being made and finally the two strains were killed by the extreme summer heat of Alabama where the writer was then located. However, in transferring the flies from generation to generation it was observed that there always remained a striking difference in the spotting of the two strains and it is believed that the effects of selection remained permanent.

Discussion of behavior of male line 501

Before discussing the genetic behavior of the various selection strains it will be well to consider an unusual type of male spotting which was found in the stocks carried. A number of stocks of flies were taken from nature at the time that the male selection lines were started. One of these stocks (line 501) was observed to produce males which had unusually large outside spots on the fifth abdominal segment (see plate 1, no. 8, page 64). The spots of this outside pair were frequently almost as large as those of the dorsal pair. This was unusual, for these spots were ordinarily much smaller than the dorsal ones. Selection was started using the most extreme males in an attempt to establish a pure

TABLE 10

Permanency of effect of selection.

A. After five generations of selection

Strain	Percent selected type	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
502 low	85	622	24	3	26	9	4	4	7		28	727
502 high	75	33	2	7	3	2	62	13	5	9	416	552

B. Same strain after seven additional generations without selection

Strain	Percent selected type	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
502 low	89	281	2		1		32	1				317
502 high	76	4			1		45	13		4	217	284

Table 10 shows the permanency of the effect of selection in line 502. Strain 502 low and 502 high originally came from the same stock. In strain 502 low selection was made for type 1 males while in strain 502 high selection was for type 16 males. (These types are shown in figure 1.) After 5 generations of selection in which considerable difference was brought about, stocks were taken from the two strains and carried unselected for seven generations. At the end it is seen that the difference in the two strains remained permanent.

stock of this type of male. Immediately an almost pure stock was obtained. Practically all of the males possessed the outside pair of spots and these spots were unusually large in most cases. Some of the males also possessed the middle pair of spots (plate 1, no. 6) making a male of the six-spotted type. Selection was carried on for several generations until practically all of the males possessed the unusual outside spots.

Genetic behavior of line 501 when crossed to strain 504 low

The 501 line was crossed with the low strain of 504. It will be remembered that the low strain of 504 was practically pure for the two-spotted condition or the absence of the outside pair of spots which were characteristic of line 501. The 501 males and females were each crossed with the opposite sex from 504 low. The F_1 results are shown in table 11. Eleven matings were made by crossing the 504 females, by the 501 males, and ten matings of the reciprocal cross. The data for these crosses show a rather striking difference in the results where the 504 female was crossed with a 501 male, as compared with the cross in the opposite direction. When the female was from 501 there was a much higher percentage of the males in the F_1 showing one or both spots of the middle pair than when the male of the same line was used. This is not a chance fluctuation but holds true for practically every mating. The middle pair of spots was not the one which was of primary interest in this set of matings for only a small percentage of the males of 501 possess the middle spots. From figure 1 it can be seen that all of the types listed in table 11, except types 1 and 11, possess one or both of the middle spots. When the 501 female was used in the cross, about thirty percent of the males in the F_1 have one or both of the middle spots, but when the 501 male was used, less than three percent of the males possessed one or both of the middle spots. The only explanation that can be offered for this peculiarity is that one or more of the genes affecting the presence of the middle spots are located in the sex chromosome. Not all of the genes for the middle spots can be in the sex chromosome, for in that case a female from 501 should transmit the character to all of her sons in the F_1 generation. After observing this tendency toward sex-linked inheritance of the middle pair of spots in 501 the writer examined some of the other data in which the middle pair of spots was involved tc

TABLE 11

Strain 501 × strain 504 low.

F_1 generation

Type of mating	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
504♀ × 501♂	373		1			1539	18	2	13	18	1964
501♀ × 504♂	244	4	8	2	1	953	90	8	105	311	1726

F_2 generation

Type of mating	Type 1	Type 2	Type 4	Type 5	Type 6	Type faint 11	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
Faint type 11♂ from cross 504♀ × 501♂	314	14	10	24	19	613	398	42	2	27	149	1612
Type 16♂ from cross 501♀ × 504♂	357	19	1	17	15	694	433	38	1	20	148	1743
Good type 11♂ from cross 501♀ × 504♂	376	13	3	9	11	667	416	26	2	14	95	1632
Total for F_2 generation	1047	46	14	50	45	1974	1247	106	5	61	392	4987

Table 11 shows the results of the cross between strain 501 and strain 504 low. Strain 501 possessed very large outside spots while strain 504 lacked them. In the F_1 results note the difference in percent of type 16 males depending on whether the male or female from 501 was used in the cross. The F_2 results indicate that it makes no difference in the results of that generation what sort of an F_1 male is used as father of the F_2 generation.

see if the same behavior had been overlooked there. A cross had been made between a strain known as the abnormal strain[4] and 502 high. In the abnormal strain the males were practically all two-spotted or lacking both in the middle and outside pair of spots, while the males of the 502 high possessed all three pairs. In the crosses between these two stocks, there was no difference in the F_1 results whether the male or female from 502 high was used. There is no indication of sex-linked inheritance with respect to the middle pair of spots, though such linkage should have been manifest in this cross since the abnormal strain was practically pure for the absence of the middle pair of spots while the 502 high was practically pure for their presence. Thus it seems necessary to conclude that there is a gene in the sex chromosome in line 501 which influences the presence of the middle pair of spots while in strain 502 high none of the genes influencing the inheritance of the middle pair of spots are in the sex-chromosome.

Turning now to the genetic behavior of the outside pair of spots for which the cross between 501 and 504 low was made, it will be seen from table 11 that in the F_1 there is a more or less incomplete dominance of the presence of the outside spots in 501 over their absence in 504 low. The count shown in the first column (type 1) of the table represents the males with neither outside spot while practically all of the other columns were for males which possessed one or both of the outside spots. As to the percentage of the males in the F_1 showing the outside spots, it seems to have made no difference whether the male or female from 501 was used in the cross. However, one difference was evident in the F_1 generation between the crosses where the 501 male or female was used in the mating. When the female of the cross was from 501 the male offspring showing the outside spots in the F_1 showed them in greater intensity than when the 501 male was used in the cross. In the cross of 501 male by 504 female, the outside spots which appeared on the males of the F_1 generation were rather faint. When the cross was made in the opposite direction many of the male offspring were almost typical 501 males with respect to the intensity of the spots. The F_2 results are also given in table 11. Three types of matings were made in the F_1 generation. From the F_1 of the cross 501 female by 504 male, both the six-spotted males and the almost typical 501 males were mated to sisters while in the F_1 of the cross 504 female by 501 male, the males showing the outside spots faintly were mated to sisters. The F_2 results show no difference between these three sorts of matings. In the table of the F_2 results the column headed type 11 represents the count of males which were typical 501 while the column just preceding it contains males with the outside spots showing faintly. The males which show the outside spots faintly are probably heterozygous while the apparently typical 501 males are probably pure, segregated males of that type. The males in the first column are typical 504 males with only the dorsal pair of spots. Some of these typical 501 and 504 males were taken from the F_2 generation and mated back to females from their respective lines. The resulting offspring were typical of the line to which the males were backcrossed showing that the two types of males used in the cross had segregated after the Mendelian manner.

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[4] The behavior of this strain is discussed later in this paper.

These crosses indicate that the outside pair of spots which are characteristic of line 501 is inherited in the usual Mendelian manner except that there is a lack of dominance of its presence or absence. There is a difference in the intensity of the outside spots on males of the F_1 generation depending upon whether the 501 male or female is used in the cross. The more intense spots appear when the 501 female is used. This might be taken to indicate that a sex-linked factor is involved in the inheritance of the outside spots. There is fairly definite evidence from the crosses made that sex-linked inheritance is involved in the transmission of the middle pair of spots in 501 although there is no evidence that the same spots in other stocks are inherited in that way.

Effect of temperature on the male spotting

During the course of the selection experiment it was fairly evident that some environmental factor was influencing the male spotting. Observations indicated that temperature was such a factor and to test this out a series of experiments were planned. These experiments were carried out at the tenth generation of selection when the divergence of the high and low strain was about as extreme as at any time during the selection experiment. The strains 500 low and high and 502 low and high were used in the tests. It had been noted that low rather than high temperature seemed to affect the results ordinarily obtained under room temperatures, so attention was directed chiefly to the effects of low temperatures as compared with room temperature.

A set of matings was made from each strain, some of them being kept under room temperatures (17°—23°C), and others being placed in a basement room where the temperatures ranged between eleven and

TABLE 12

Effects of temperature on spotting of males.

Temperature condition	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
A. Strain 500 low											
Room (17-23° C)	546	5	2	6	8	9				3	579
Basement (11-15° C)	69					418	6		3	4	520
B. Strain 500 high											
Room (17-23° C)	99	10	12	23	28	49	23	6	24	422	696
Basement (11-15° C)						54	10		15	73	152
C. Strain 502 low											
Room (17-23° C)	92		1	4	1	166	7	1	4	8	284
Basement (11-15° C)	4					249			1		254
D. Strain 502 high											
Room (17-23° C)									1	502	503
Basement (11-15° C)						15	8		5	97	125
Incubator (25° C)									1	61	62

In table 12 are shown the results of a series of experiments to test the effect of temperature on the spotting of males. Under room temperatures strain 500 low was almost pure for type 1 males, while strain 502 high was almost pure for type 16 males. Strain 500 high gave a fairly high percent of type 16 males under room temperatures while strain 502 low gave a good percent of type 1 males. From the tables it will be seen that the lower temperatures caused an increase in the type 11 males or in other words they brought out the outside pair of spots in all strains in which it did not already appear.

fifteen degrees centigrade. The species will not breed under temperatures much lower than those in the basement. The results are shown in table 12. No effects of high temperature had been observed in the laboratory, but to test the effect of high temperatures one culture of 502 high was reared in an incubator where the temperature was kept at 25°C. Strain 500 low which at room temperature produced almost exclusively males of the two-spotted type, reacted quite differently at lower temperatures. When 500 low was reared at the basement temperatures a large percentage of the males possessed very distinct outside spots. The middle pair of spots was not affected by the low temperatures. Strain 500 high which at room temperatures ordinarily produced a large majority of six-spotted males, at basement temperature produced no two-spotted males at all, but only males having the outside pair of spots. Strain 502 low never produced as high a percentage of two-spotted males as the other low strains. This strain proved to be always more susceptible to the effect of low temperatures. The count for room temperature in table 12 is hardly characteristic of the strain, for at that time the percentage of two-spotted males was somewhat low. In the stocks of 502 low reared at the basement temperatures practically all of the males were those possessing the outside pair of spots. The stock of 502 high which at the time of the test was practically pure for six-spotted males when reared at room temperatures was also reared in the basement room. Since this stock was, at room temperatures, already pure for six-spotted males, low temperatures could not increase the percentage of males possessing the outside pair of spots. The results show that the low temperatures had no effect on the outside pair of spots but did seem to reduce somewhat the number of males possessing the middle spots. Instead of being pure for six-spotted males as was the condition under room temperatures a few of the males had one or both of the middle spots missing. Strain 502 high was also subjected to high temperatures. It was reared in an incubator where the temperature was kept at 25°C. Table 12 shows that results obtained at this temperature were not different from those obtained under room conditions.

These tests show that the outside pair of spots could be brought out by low temperatures regardless of the spotting condition of the strain induced by selection. Even in lines where none of the males under room temperatures possessed the outside spots, these spots could be made very distinct in a large majority of the males under lower temperatures. In strains where the males did not possess the middle pair of spots under room temperatures, the lower temperatures did not bring out these spots as it did the outside pair. Strains which under room temperatures possessed the middle pair of spots showed a tendency to lack them at low temperatures. In fact the lower temperatures showed a slight tendency to reduce the distinctness of this pair of spots. The lower temperatures also had a tendency to intensify the general pigmentation of the fly. Temperatures higher than room temperatures seemed to have no effect upon either the middle or outside pair of spots. It is true that the data on the effects of higher temperatures are rather meager and more matings would have given more conclusive results. It was the intention to test this out further at a later date but pressure of other work has made this impossible. However, judging from observations made during the course of the selection experiment, I feel confident that more extensive data would only substantiate the results already recorded.

The facts brought out by this experiment have a definite bearing upon the selection experiments. Low temperatures occasionally encountered in the laboratory would have considerable influence upon the results of the selection experiment. This effect, especially upon the low strains, was noted several times. The effect of low temperatures is responsible for some of the irregularities in the graphs representing the behavior of the strains (see figures 2, 3 and 4). The rather sudden upward divergence of the graphs for the low strains near the end of the selection experiments can be correlated with a period of low temperatures which decreased the percentage of two-spotted males.

Crosses between the various male strains

At the end of the selection experiment when the various male strains had become fairly well established, crosses were made between some of them. A large series of experiments were planned but only a limited number were carried out because of the interference of other duties. Two types of matings were made, one set in which the high and low strains of the same line were crossed and the other in which the high strains of two different lines were used.

For the set of matings where the high and low strains of the same line were crossed, line 500 was used. Generation fifteen of the 500 low was crossed with generation fourteen of strain 500 high. The cross, 500 low male by 500 high female, and its reciprocal were both made and the results from the two types of crosses were the same (table 13). As to dominance or recessiveness, it would hardly be expected that either strain would act as a complete dominant for neither was absolutely pure for its type. The F_1 results show a high percentage of the males to be of the two-spotted type, but there are also considerably more six-spotted males than were to be found in the low strain. So the results show spotting in the males which is intermediate between the two strains. The lack of Mendelian ratios is probably due to the lack of dominance of either type. This would affect not only the F_1 generation but also the ratios obtained in the F_2 generation.

For the cross between two high strains, lines 500 and 502 were used. The fourteenth generation of the 500 high was crossed with the same generation of the strain 502 high. At the time of the cross the 502 high

TABLE 13

F_1 generation of crosses between various male strains.

A. Strain 500 high × strain 500 low

Type of cross	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
500 high ♀ × 500 low ♂	245	17	1	20	12	34	10		1	59	399
500 low ♀ × 500 high ♂	465	17	1	18	11	47	8		5	40	612
Total	710	34	2	38	23	81	18		6	99	1011

B. Strain 502 high × strain 500 high

502 high ♀ × 500 high ♂	5	2			1	14	5			425	452
500 high ♀ × 502 high ♂	4			1		3	1		1	136	146
Total	9	2		1	1	17	6		1	561	598

The results in table 13 are for the crosses between the high and low strains of line 500 and between the high strains of lines 500 and 502. The condition of the two strains of line 500 at the time of this cross is shown in the last generation of selection of these strains (tables 1 and 2). The cross gave a result which was intermediate between the two strains. The cross between the two high strains gave a result which might be considered like either strain since the two strains were very similar at the time of the cross.

strain was practically true for the six-spotted type of males while strain 500 high still threw a few two-spotted males in each generation. The cross was made in both directions with no difference resulting between the two types of crosses. The results found in table 13 show a very high percentage of six-spotted males. The results which are about characteristic of either strain show that the same factors are responsible for the spotting character in each strain.

Summary

1. Selection was effective in changing the percentage of two-spotted or six-spotted males in a strain. There was a difference in amenability to selection of the different lines taken from nature.

2. The effects of selection remained permanent, at least for several generations.

3. The crosses with the 501 type of male indicate that there is sex-linked inheritance involved in the case of both the middle and outside pairs of spots.

4. Low temperatures tend to bring out the outside pair of spots on the male and also seem to have a slight tendency to cause the middle pair of spots to disappear.

5. Neither type is dominant in the F_1 generation when strains with two-spotted males are crossed to strains with six-spotted ones.

6. The results of crosses between the high strains of different lines indicate that their spottedness is due to the same factors.

FEMALE SPOTTING

Abnormal female

While examining flies on March 16, 1916, in one bottle, six females were found which had the middle pair of spots missing from the fourth abdominal segment. The other two pairs of spots were normal in size and position but there was no indication of the middle pair (plate 1, no. 5, page 64). These females were mated to brothers and from this mating originated a strain of flies which was called the "abnormal female" strain or for the sake of brevity the "ab" strain. This strain is characterized by the absence of the middle pair of spots from the fifth abdominal segment.

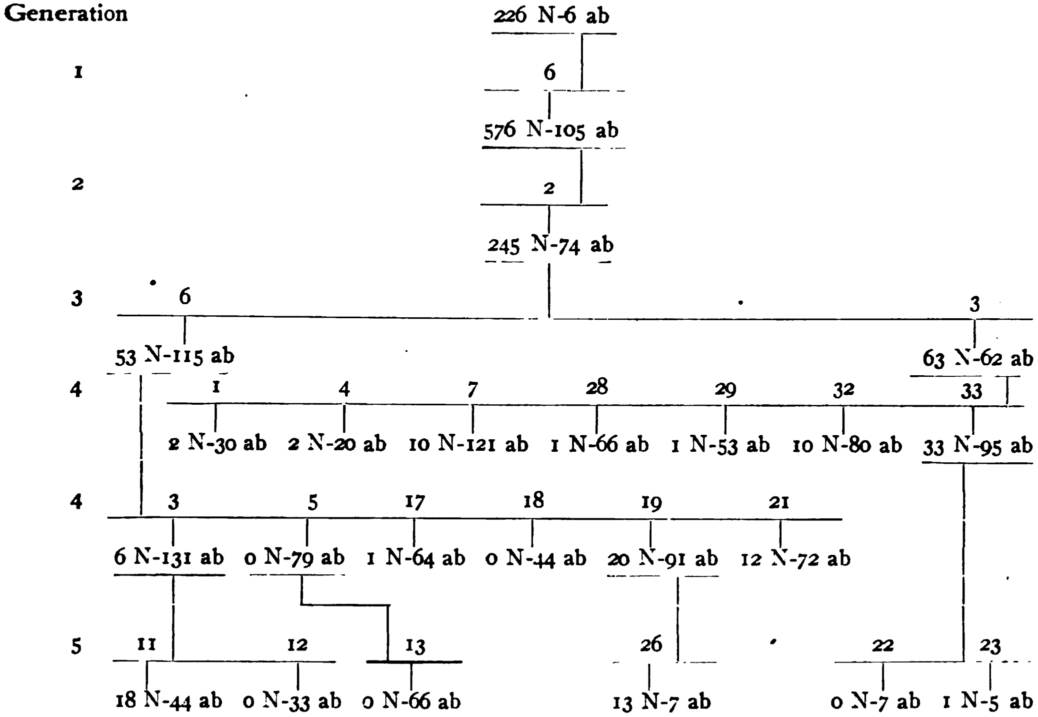
Since there is sexual dimorphism in the spotting of the fifth abdominal segment, the problem of analyzing the spotting inheritance became rather difficult. In mating the original ab females to brothers, the males had to be selected blindly, as there was no way of knowing what relation if any, the male spotting bore to the female spotting. So in selection for purity with respect to the ab condition, the type of males used was kept constant, only those being used which possessed but one pair of spots, the large dorsal ones (plate 1, no. 1).

Selection of ab strain

As already mentioned the six original ab females were mated to two-spotted brothers in stock culture and the total of their offspring showed 576 normal females and 105 ab females. (It might be said here that these early counts of ab females are not absolutely accurate as there are always a few flies which have the ab spotting on one side and normal on the other. These intermediates at first were called ab or normals depending upon the intensity of the spots. Later they were recorded in the counts.) It was hoped that by mating ab females to their brothers a pure strain, with respect to the ab female, might be established. At first the results seemed rather puzzling for in each cross ab females were

TABLE 14

Selection from the original abnormal females to establish a pure abnormal strain.



The letter "N" in table 14 indicates normal females and "ab" stands for abnormal females. The figures above the line indicate the mating number in the generation. This selection line was lost at the end of the fifth generation.

obtained but the relative number of ab and normal females was quite variable. Table 14 shows the selection which was carried on for five generations. It will be seen that in the fourth generation of selection there were two matings (matings 5 and 18) which gave only ab females. Similar results were obtained in the fifth generation. Unfortunately at this time the selected strain was lost, due to the extreme summer heat. Previously, however, the ab strain had been crossed to a wild strain which did not show the ab condition in the females. From the F_1 of this cross a few ab females were saved and mated to brothers and in this way the selection was renewed.

It should be noted that the cross from which the ab strain was regained was one in which a male from the ab strain had been mated to a normal female from a wild strain (recorded as original cross in table 15).

Although the male himself did not show the ab character, he was capable of transmitting it to his offspring. In the F_2, or generation B, a large number of flies were obtained and the selection for a pure ab strain was once more started. In the third generation eighteen matings were made with the ab females as mothers, and in only one case (mating C 8) were the females obtained all ab and this was probably due to chance as the count was very small. In generation D the ab females were used in twenty-nine matings and six of them proved to be pure with respect to the ab condition. It was then thought that the strain had reached purity for the ab condition and thirteen matings were made from among the offspring of the matings which bred true. The results of this generation were again disappointing as the thirteen matings did not all breed true. Four matings were made from D27 and they were all pure, five were made from D44 and none of them were pure, four were made from D45 and two were pure. These inconsistencies could not be explained at this time but later matings added new light which made them explainable. From among the offspring of D27 more matings were made all of which bred true again. This is the origin of the ab strain which has bred true for many generations.

While the strain producing the ab females was being selected for purity, back selection was also attempted. This was to see if selection was effective in both directions. From mating B1 (table 15) back

TABLE 15

Selection in strain producing abnormal females.

Generation	Parent	Female spotting				Generation	Parent	Female spotting			
		Normal	Ab	Left	Right			Normal	Ab	Left	Right
Original cross		10	9			E7	D2	1	135	3	5
						E8	D15		130		1
A2		6	7			E11	D15	22	171	5	5
B1	A2	200	138	34	31	E13	D30		17		
C3	B1	51	92	18	11	E15	D31		117		
C4	B1	46	48	8	5	E16	D44	8	94	10	4
C5	B1	70	36	6	11	E17	D27		69		
C6	B1	16	65	9	10	E18	D27		124		
C7	B1	105	30	10	6	E20	D27		126		
C8	B1		5			E21	D27		128		
C10	B1	59	47	10	7	E23	D44	13	62	2	3
C12	B1	1	14	2		E24	D45	57	165	5	7
C13	B1	51	13	3	1	E27	D15	7	349	12	11
C16	B1	8	16	2	1	E28	D30		135		
C17	B1	55	9	8	2	E29	D30		125		

TABLE 15 (continued)

Generation	Parent	Female Spotting				Generation	Parent	Female spotting			
		Normal	Ab	Left	Right			Normal	Ab	Left	Right
C18	B1	54	20	5	4	E30	D30		115		2
C19	B1	24	33	7	5	E33	D33	1	179		1
C21	B1	2	11	2	2	E34	D33	4	153	2	
C22	B1	26	33	7	4	E36	D44	24	159	6	12
C26	B1	9	69	6	6	E37	D44	31	155	9	3
C27	B1	3	14	1		E39	D44	2	251	1	
C32	B1	2	18	1	1	E40	D45	1	237		1
D2	C1	8	81	4		E41	D45		198	1	
D3	C4	10	26	5	3	E42	D45		63		
D4	C4	39	67	3	9	E47	D4	27	242	8	7
D6	C4	29	46	6	3	F1	E18		161		
D7	C3	46	16	2	4	F2	E20		176		
D8	C3	17	44	4	7	F3	E20		84		
D9	C3	26	14	2	3	F4	E20		82		
D11	C6	2	16	4		F5	E20		175		
D12	C5	14	14	2	3	F6	E28		222		
D13	C5	25	30	4	2	F7	E28		76		
D15	C6	43	50	6	11	F8	E28		254		
D16	C7	3	41	2	2	F9	E28		178		
D17	C3	3	64	1	2	F11	E41	1	316	4	2
D18	C4	7	37	4	2	F13	E41	37	192	8	7
D22	C12		81	1		F16	E25	66	251	19	13
D26	C8	25	250	7	13	F17	E37	6	133	5	5
D27	C12		98			F18	E37	2	106		1
D30	C12	1	124			F19	E37	15	83	7	3
D31	C8	7	16	3		F25	E25	10	48	4	2
D33	C12	9	23	2	3	F26	E24		240	2	
D37	C6		27		1	F29	E24	84	159	22	16
D38	C6	18	36	3	3	F30	E41	15	207	12	3
D44	C26		253	1		F31	E29		414		1
D45	C26		138	2		F32	E21	2	370		
D46	C32		93			G1	F2		195	1	
D47	C32	10	31	2	1	G2	F2		176		
D49	C32	16	37	4	6	G3	F8		401		
D50	C26	9	145	1	1	G4	F2		66		
D51	C32	5	30	1	2	G5	F8		100		
E1	D15	10	285	8	12	G6	F2		128		
E2	D15	14	288	11	13	G7	F8		59		
E3	D15		247	1		H1	G3		186		
E4	D2		57			H2	G3		198		
E5	D2	1	4		2	I1	H1		67		
E6	D2	3	59	2		I2	H2		214		

Table 15 shows the selection required to bring the abnormal strain to purity. The letters in the column to the left indicate the generation of selection and the figures which follow them indicate the mating number in the generation. "Left" indicates a female which is abnormal only on the left side and "right" one which is abnormal on the right side.

TABLE 16

Back selection of abnormal strain.

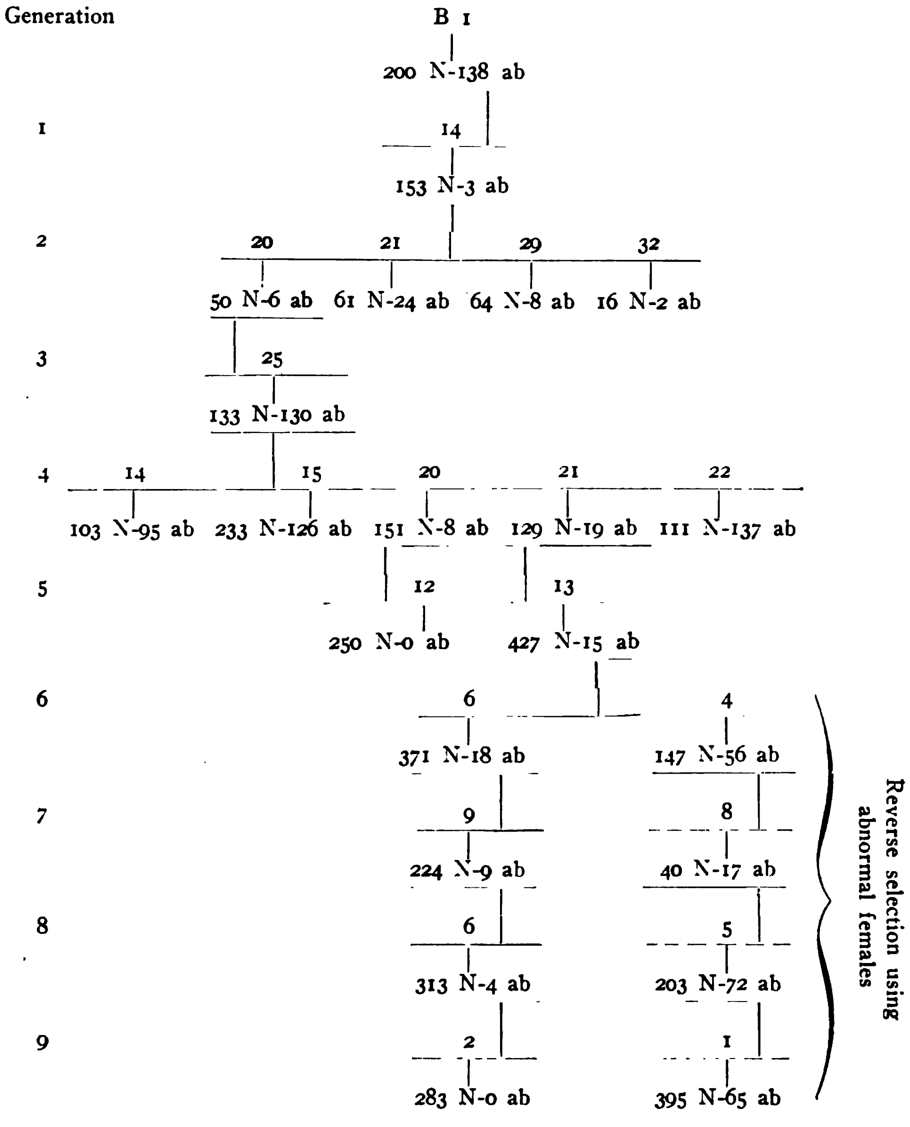


Table 16 gives the results of selection for the normal female in the strain producing a high percent of abnormal females. The selection here is in a direction opposite to that in table 15 but started with mating B1 of that table. At generation 6 selection was again reversed (selecting for ab females) and is shown as the offspring of mating 4 of generation 6. The selection for normal females was still continued and is shown as the offspring of mating 6 of generation 6.

selection was started, using normal females. At the time back selection was started the strain was throwing about an equal number of ab females and normal females. Selection of this sort was carried on for nine generations and the strain became practically pure for normal females (table 16). In the fifth generation of back selection when the strain was approaching purity for the normal females, selection was started again in the reverse direction. This was done by using ab females as mothers. Selections in opposite directions were carried along for four generations, by which time one strain had become practically pure for the normal female, while the other was again throwing a large percentage of ab females. These experiments show that selection is effective in either direction before purity is reached and that purity may be reached for either type if selection is carried far enough.

The question as to how the ab strain originated,—whether as a simple mutation from the beginning, or as a result of selection—will not be discussed at present. Before taking up this question we will examine the results of the matings of this strain to normal strains. These matings were made in attempting to analyze this variation.

Matings of the ab strain with the normal strain 500 low

The strain 500 low to which the ab strain was crossed was one which had been bred in the laboratory for six generations and was in no way related to the ab strain. The males of this strain were almost all of what may be called the normal male type, having only the two large spots. The 500 low strain had been selected for three generations for the above type of male and from the beginning showed a tendency to produce males of that sort. The females of the 500 low strain were all normal, that is, having a full set of six spots. In all strains bred in the laboratory a few ab females appeared, but they seemed to be somatic variations as will be shown later (page 102). Since this strain was very similar to the ab strain, as far as the male spotting was concerned, and normal with respect to the female spotting, it was considered a good strain with which to cross in testing the genetic behavior of the ab strain.

As will be seen in table 17 twelve matings were made between abnormal females and males of the normal strain, and nine matings were made between males of the abnormal strain and females of the normal strain. For the first type of cross the totals were 977 normal females, 1645 ab females, 329 "left" females and 273 "right" females.[1] In the

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[1] By "left" female is meant a female which is abnormal only on the left side and the expression "right" refers in the same manner to the right side.

TABLE 17

F_1 generation of cross abnormal strain × normal strain 500 low.

Abnormal ♀ × normal ♂					Normal ♀ × abnormal ♂				
Mating number	Female spotting				Mating number	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
150	57	158	17	17	157	164	190	42	46
151	64	81	30	19	158	204	206	56	39
153	15	142	19	15	159	35	91	21	21
154	72	124	28	21	168	66	177	27	32
155	79	187	25	29	169	123	329	51	59
162	24	103	14	13	170	75	261	43	42
163	57	77	17	11	171	24	158	13	10
164	53	92	22	24	172	52	183	30	22
165	91	26	13	8	250	154	230	60	55
166	92	139	33	26					
188	281	227	71	49					
251	92	289	40	41					
Totals	977	1645	329	273	Totals	897	1833	343	326

Grand total, 1874 normals, 3478 abnormals, 672 lefts, 599 rights.

In table 17 are shown the F_1 results of a cross between the abnormal female strain and strain 500 low. The females in strain 500 low were all normals. Crosses in both directions are shown. Both normal and abnormal females appeared in the F_1 generation with about twice as many abnormals as normals.

second set of matings in which the male from the ab strain was used the totals were 897 normals, 1833 abnormals, 343 lefts and 326 rights. These matings were all single-pair matings. The total of the F_1 is 1874 normals, 3478 abnormals, 672 lefts and 599 rights. These F_1 results threw very little light upon the nature of the abnormal variation. They do show however that either the male or the female of the ab strain will transmit the ab character even though the males do not show it. It is also seen that the ratio of abnormals and normals is about the same regardless of which way the cross was made.

In order to carry the analysis still further F_2 matings were made (table 18). Since there were both ab and normal females in the F_1 it was necessary to make two types of matings in the F_2, that is, ab females with brothers and normal females with brothers. The totals for the first type of matings were 945 normals, 1248 abnormals, 138 lefts and 112 rights, and for the second type 1879 normals, 1415 abnormals, 167 lefts and 159 rights. If the ab condition acts as a Mendelian character there should be no difference in the genetic constitution of the two types of females in the F_1. Then F_1 ab and normal females when crossed should behave the same with respect to the sort of offspring to which they give rise. Three types of crosses were made in which the behavior of these two sorts of females can be compared: (a) F_1 females back-crossed to the pure ab strain, (b) F_1 females back-crossed to the normal strain which was used in the cross, and (c) F_1 females by brothers. The totals for the results of these crosses are shown in table 19. Considering the individual matings the results seem quite variable in each type of mating, but the totals show evidence of a slight difference in behavior of the two types of females appearing in the F_1. Where the two types of females were back-crossed to the pure ab strain there is a higher relative number of females, as compared with the number of normals produced when the ab female was the mother. Where the cross was a back-cross to the normal strain there was no apparent difference between the two females, but again when they were crossed to their brothers

TABLE 18

F_2 generation of cross abnormal female strain × strain 500 low.

Abnormal F_1 ♀ × normal brother					Normal F_1 ♀ × normal brother				
Mating number	Female spotting				Mating number	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
154 A1	80	125	20	13	154 A3	164	177	10	13
154 A2	73	177	5	9	157 A1	114	137	18	22
157 A3	167	157	27	20	157 A2	178	200	12	21
157 A4	126	101	15	8	157 A5	166	113	11	5
162 A1	107	179	15	16	157 A6	188	156	24	15
164 A3	63	172	14	11	164 A1	101	72	11	4
164 A4	105	213	19	16	164 A2	87	146	9	14
169 A1	83	28	5	5	169 A4	128	89	10	10
169 A2	141	96	18	14	188 A1	262	148	33	17
					250 A1	317	88	13	20
					251 A1	174	89	16	18
Totals	945	1248	138	112	Totals	1879	1415	167	159

Grand total, 2824 normals, 2663 abnormals, 305 lefts, 271 rights.

In table 18 showing the F_2 results of the cross abnormal female strain by strain 500 low, the grand total shows about an equal number of abnormal and normal females. However the ab F_1 female gave a higher percentage of abnormals in the F_2 than the normal female from the same generation.

a higher relative number of ab females appeared when the maternal parent was an ab female. With the large number of individuals examined in these crosses it hardly seems possible that these variations could be due to chance. Assuming that ab is an ordinary Mendelian character, these two types of F_1 females should react the same genetically, but when they are each crossed to males of the same type, they each tend to beget young which show a higher percentage like their mothers. Although there is a variation in their behavior the preponderance is never large enough to obliterate any class of individuals which should have segregated out. The factor which brings about the difference seems to play entirely upon the heterozygous individuals and therefore does not prevent the ab condition from still being considered as behaving in a Mendelian manner.

TABLE 19

A comparison of the behavior of the two types of females appearing in the F_1 generation.

Type of male used in cross	F_1 ab female				F_1 normal female			
	Female spotting				Female spotting			
	Normal	Ab	Left	Right	Normal	Ab	Left	Right
Male from pure normal strain	2706	382	142	138	2812	486	133	127
Male from pure ab strain	203	2922	110	81	223	1588	103	81
F_1 brother	945	1248	138	112	1879	1415	167	159

Table 19 shows the results of three types of crosses comparing the behavior of the two types of females found in the F_1 generation. In two of the crosses the F_1 ab female produced more abnormals than her normal sister.

The F_2 normal and ab females were back-crossed to the respective strains from which they came, to test whether there was Mendelian segregation. These results are shown in table 20. Where the F_2 ab female was back-crossed to a male from the ab strain, eight of the fifteen matings were pure for the ab condition. Where the F_2 normal female was back-crossed to a male from the normal strain, nine out of the fifteen matings bred practically pure for the normal condition. The same sort of back-crosses were made in the F_1 generation (see table 19). Out of ten crosses between F_1 ab females and males from the ab strain no matings went pure for the ab condition and likewise no all-normal counts were obtained from making eight crosses between F_1 normal females and males from the normal strain.

TABLE 20

Back crosses of the two types of females appearing in the F_2 generation.

F_2 ab ♀ × pure ab ♂					F_2 normal ♀ × pure normal ♂				
Mating number	Female spotting				Mating number	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
205		141			210	398	10	10	14
206	19	109	2	3	211	56		4	2
207	22	94	9	7	212	321	10	7	15
208		157			213	387	5	6	4
209		39			214	220	21	9	10
220	14	222	10	4	218	208	57	18	12
221		67			225	369			
222	3	61		1	226	314		1	
223	17	157	9	7	227	227	1	1	3
224		223			228	269	49	18	15
241		86	1		229	210		1	
256	43	251	5	10	263	361	1		1
266	20	268	9	17	264	336		1	1
269		80			265	278	2	4	3
270		113			267	263		1	1

The crosses in table 20 were to test the segregation of the normal and abnormal females in the F_2 generation. Ab females from the F_2 generation were back-crossed to males of the pure ab strain while normal females of the same generation were back-crossed to males of the pure normal strain. Of the first cross, eight out of fifteen of the females acted as pure abnormals and in the latter cross, nine out of fifteen of the females acted as pure normals.

The fact that pure stocks were regained by back-crossing the two types of F_2 females to the stock from which they originated, indicates that there is Mendelian segregation. That this could not be done with apparently the same types of females from the F_1 also shows Mendelian behavior.

Some of the F_2 females having a spot on one side only (recorded as lefts or rights) were tested as to their genetic constitution. The test was made by back-crossing them to the original strains from which they came. The number of such crosses was not large but in all of them both abnormal and normal females appeared, showing that the females used were heterozygous flies. So the flies indicated in the tables as lefts and rights are probably individuals which are heterozygous for the ab and normal condition.

Summary of various types of matings in testing the behavior of ab when crossed to the strain 500 low

1. In the F_1 generation resulting from the cross between the ab female and the 500 low strain and its reciprocal, there is not a dominance of one or the other of the contrasted characters. Both types appear in F_1 with about twice as many abnormals as normals. Both males and females from the ab strain are capable of transmitting the ab character. although only the females show it.

2. The two types of F_1 females when back-crossed to their respective original strains did not give pure stock, showing that these females were heterozygous and there was no segregation in this generation.

3. The two types of F_1 females gave slightly different ratios when crossed to males of the same genetic constitution. The F_1 ab female gave more abnormals than the F_1 normal female when they were crossed to the same male.

4. There was Mendelian segregation in F2 of the two original types. This was shown by back-crosses of the two types of females in F_2 and results of the F_2 generation.

Crosses between the ab strain and strain 502 high

In an attempt to further analyze the genetic behavior of ab, the ab strain was mated to strain 502 high. The strain 502 high was like 500 low so far as the female condition was concerned, that is, all the females were normal, i.e. with a full set of six spots. The only difference between these strains was that the males in strain 502 high had been selected for a full set of six spots while the strain 500 low had been selected for the normal or two-spotted male condition.

To the writer's surprise entirely different ratios were obtained (table 21) in F_1 when the ab strain was crossed to the strain 502 high. With apparently the same environmental conditions as before, a large majority of the F_1 flies were normal. There was practically a dominance of the normal condition over the ab. Out of the 1965 F_1 females examined only 71 were abnormal.

These F_1 results are quite different from those obtained when ab was crossed to strain 500 low (table 17). The count for the F_1 generation of the cross of ab with 500 low was 1874 normals, 3478 abnormals, 672 lefts and 599 rights. For the abnormal-by-502-high cross the counts were 1756 normals, 71 abnormals, 69 lefts and 69 rights. Since there is almost complete dominance of the normal condition over the abnormal condition in crossing ab by the 502 high strain, it would be expected that

TABLE 21

Totals of the F_1 and F_2 generations of the cross abnormal strain × strain 502 high.

F_1 generation

Female spotting			
Normal	Ab	Left	Right
1756	71	69	69

F_2 generation

3169	1117	54	47

The counts in table 21 are the results of the F_1 and F_2 generations of the cross between strain 502 high and the abnormal female strain. Very few abnormals appeared in the F_1 generation and an approximate 3:1 Mendelian ratio was obtained in the F_2 generation. Note that the ratios in this cross are quite different from those obtained in the cross, abnormal female by strain 500 low.

the usual 3 to 1 ratio would be obtained in the F_2. This was found to be true as will be seen in table 21. The totals for the F_2 were 3169 normals, 1117 abnormals, 54 lefts, and 47 rights. F_2 data were obtained from ab female by 502 high male and the reciprocal cross, but the ratios were approximately the same in each.

F_3 matings were also made to see whether a pure ab strain might be extracted. Fourteen matings were made of which two bred true to the ab condition. Since there is almost complete dominance of the normal condition, probably all of the ab females obtained in the F_2 are pure abnormals. There is no way to determine the genetic constitution of the males with respect to the ab condition except by breeding. One-fourth of the males should be pure for the ab condition, so one-fourth of the F_3 matings should have yielded only abnormals. Only two out of fourteen matings were of this character, but this shortage was probably due to chance because of the small number of matings.

Here we have the ab condition acting very much as an ordinary Mendelian character, the normal condition being almost completely dominant in the F_1 generation. The only thing unusual about this set of matings was that there was such a difference in behavior of the ab character here as compared with its behavior when crossed to strain 500 low. The explanation for this is found in the results of the tests which follow.

Genetic behavior of the ab strain when crossed to various strains

Since such a difference was found in the F_1 behavior of the ab strain when crossed to strains 500 low and 502 high, it was decided to test its behavior with all of the strains carried. The strains used were the selection strains of the experiment upon the male spotting. The females in all these strains were normal, having a full set of six spots. The only difference was that part of the strains had been selected for males with two spots while the rest were selected for the six-spotted condition of the males. When these matings were made the high strains had attained a condition in which a large percentage of the males were of the desired type with six spots, and likewise the low strains possessed mostly two-spotted males. The matings were all made at once and subjected to as nearly identical environmental conditions as possible. When the F_1 counts were made the same difference was again found to exist between the ratios when ab was crossed to 500 low and 502 high. Some other rather striking results were obtained from this series of crosses.

The results show that the dominance or recessiveness in the F_1 is affected by the degree of spottedness of the males in the strain in which the ab strain is crossed (see table 22). All three of the strains which are called high give almost complete dominance of the normal female condition over the ab in F_1 while the low strains crossed to the ab strain yield nearly half or more of abnormal F_1 females. The ratios obtained were as follows: In line 500 the low strain produced 307 normals to 289 abnormals, and the high strain 710 normals to 44 abnormals; in line 502 the low strain, 176 normals to 259 abnormals, and the high strain, 457 normals to 7 abnormals; in line 504 the low strain, 166 normals to 427 abnormals, and the high strain, 296 normals to 93 abnormals. Since this consistent difference appeared in the reaction of the high and

TABLE 22

Results of F_1 generation in crossing abnormal strain with male-selection strains.

Strain crossed with ab	Female spotting				Strain crossed with ab	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
500 low	370	289	60	72	500 high	710	44	28	18
502 low	176	259	23	24	502 high	457	7	12	8
504 low	166	427	37	45	504 high	296	93	20	15

Table 22 shows the difference in reaction of the abnormal female strain in the F_1 generation when crossed to the various strains of the male selection lines. The percent of ab females is much higher in crosses to the low strains than in the crosses to the high strains.

low strains in crosses to the ab strain when all environmental factors were kept constant, it seems that the difference in genetic constitution of the two strains is the responsible factor.

Relation of temperature to genetic behavior of abnormal female condition

After the ab strain became once established it was quite evident that variations of temperature had no effect upon it, for although there was considerable fluctuation in the temperature of the laboratory the strain remained pure for the ab condition.

The variability of the recessiveness or dominance of the ab in the F_1 led the writer to suspect that some environmental condition might be playing a part. In addition to the influence of the "spottedness" of the males in the strain to which it was crossed there seemed to be some other factor. When the type of male to which the ab was crossed was kept constant there was still considerable variation in the F_1 results. This was most noticeable in crosses where strains possessing normal or two-spotted males were used, resulting in F_1 ratios with a high percentage of ab females. Here were obtained rather variable results. Temperature being one of the simplest environmental factors to analyze, a series of experiments were carried out to test its influence upon the behavior of ab in the F_1 generation.

In testing the effect of temperature, flies reared at room temperature were compared with some reared at high temperature in an incubator, and also with others reared at a low temperature in a basement room without heat. The effects of temperature upon the F_1 behavior of ab when crossed to strains 500 low and 502 high were tested. The results of these two experiments are given in table 23. The only place where it was possible to keep the temperature constant was in the incubator. The laboratory and basement temperatures were somewhat variable but the range of variation was sufficiently different to make a satisfactory experiment. The incubator was kept at 25°C which was just about the maximum temperature at which it was possible to rear *Drosophila busckii*. Even at that temperature the flies were somewhat undersized and reduced in number. Immediately after pairing the flies, the culture bottle was placed in the incubator and kept there continually except while the progeny was being examined. In the case of the cold, the flies were also reared at about the minimum temperature at which they would reproduce. The basement-room temperatures were so low that it was difficult to get the flies to mate when kept there from the beginning. To overcome this the flies

TABLE 23

Effect of temperature on the F_1 behavior of the abnormal strain.

F_1 results of cross ab × 500 low					F_1 results of cross ab × 502 high				
Temperature condition	Female spotting				Temperature condition	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
Room 17-23° C	370	289	60	72	Room 17-23° C	1299	64	57	61
Incubator 25° C	466	189	71	66	Incubator 25° C	142	1	2	4
Cold 11-16° C	3	625	8	4	Cold 9-15° C	265	133	23	42

Table 23 shows the effect of temperature on the F_1 behavior of the abnormal strain when crossed to strains 500 low and 502 high. It has already been shown that the ab strain reacts differently with each of these strains in the F_1 generation under normal room temperatures. From the table it is seen that lower temperatures increase the percent of ab females in the F_1 generation in each of the crosses.

were started in the laboratory and kept there till several eggs were laid. In the cross between 500 low and ab, in the low-temperature series, the culture bottles were kept at laboratory temperatures for the first week and then kept in the cold for the rest of the time. At that time the basement temperature varied between eleven and sixteen degrees centigrade. When the experiment was carried out with the strain 502 high the basement temperatures were somewhat lower (9°—15°C) and it was necessary not only to start the cultures in the laboratory but also to alternate cultures between the laboratory and cold room.

The results obtained from these matings are as follows: High temperatures tend to increase the number of normal females in the F_1 generation while low temperatures tend to increase the number of abnormals. As will be seen in table 23 giving these results, the temperatures seem to have a greater or less range of effect. Under room temperatures, strains 502 high and 500 low, due to a difference in spottedness, reacted differently in the F_1 generation when crossed with ab. Under the different temperature conditions they still retained their characteristic influence upon the F_1 behavior of ab. The lowest temperature used made the ab condition almost completely dominant when crossed to 500 low while the highest temperatures used made the ab condition almost completely recessive when crossed to 502 high. This is a very pretty demonstration of the combined influence of two factors upon the behavior of a Mendelian character.

Since temperature influences so much the ratios of the F_1 generation it is evident that its influence is upon heterozygous flies. To further demonstrate this some bottles of the F_2 generation of the cross 502 high by ab were reared in the cold. In this case a larger percentage of the females should be of the ab type than when the F_1 generation of the same cross was reared under the same condition. In the F_2 generation some pure abnormals should segregate out and not being influenced by the low temperature would increase this class. The results of the F_2 generation reared under the low temperatures were as follows: 398 normals, 398 abnormals, 18 lefts and 17 rights. The results of the F_1 generation reared under the same conditions were (table 23); 265 normals, 133 abnormals, 23 lefts and 42 rights. It is seen that the expected increase in the percentage of abnormals in the F_2 generation occurred. This is additional evidence that temperature in modifying the ratios obtained from crosses of the ab female strain, acts upon the heterozygous flies.

Linkage

Since the writer (WARREN 1917) had earlier found two eye mutations in this species it was thought that crosses between these two mutations and ab might be of interest. Earlier crosses between these two eye mutations indicated that they were probably in the same chromosome. The two eye mutations had been called "red" and "chocolate."

Ab was first crossed to the mutation red eye. In the F_1, as had been observed in other crosses, the ab was only partially recessive to the normal spotting in the red-eye strain. In the F_2, in case the red-eye mutation and ab were in the same chromosome we would expect to get no red ab females (the males cannot be considered since they do not show the ab character). If the two mutations should not be in the same chromosome we would expect independent segregation of the two characters in the F_2 and the chances would be that about one-half of the red-eyed flies would be abnormals. The F_2 results (table 24) were as follows: 193 red eye, normal-spotted; 110 red eye, ab-spotted; 640 normal eye, normal-spotted; 472 normal eye, ab-spotted. So it is seen that there is no linkage between red eye and ab, or in other words they are not in the same chromosome.

Ab was also crossed to chocolate eye. Since the red- and chocolate-eye mutations had been shown to be probably in the same chromosome we would expect ab to behave in the same manner when crossed to chocolate as it did with red eye. This expectation was realized, for chocolate and

TABLE 24

Crosses testing linkage between the abnormal character and two eye mutations.

A. F_2 of red-eyed strain × abnormal strain

Red-eyed females				Normal-eyed females			
Normal	Ab	Left	Right	Normal	Ab	Left	Right
193	110	12	12	640	472	47	34

B. F_2 of chocolate-eyed strain × abnormal strain

Chocolate-eyed females				Normal-eyed females			
Normal	Ab	Left	Right	Normal	Ab	Left	Right
102	121	14	10	319	398	37	47

Table 24 gives the results of crosses to test the linkage between the abnormal character and two eye mutations. Since there was independent segregation of the two characters in both crosses it can be assumed that the factor for the ab character is not in the same chromosome as the two eye mutations.

ab segregated independently in the F_2 generation. The results were as follows: 102 chocolate eye, normal-spotted; 121 chocolate eye, ab-spotted; 319 normal eye, normal-spotted; 398 normal eye, ab-spotted. So the indications from these crosses are that the mutation for the ab condition was not in the same chromosome in which the red and chocolate eye occurred. Since no other mutations have been found in this species it was impossible to test the linkage further.

Attempts to establish an ab strain from other stocks

In practically every strain of flies which was carried any length of time there appeared an occasional female which had the ab type of spotting. In some cases, out of a day's count of sixty females from a bottle there might be four or five females with the ab type of spotting. So high a percentage was unusual, for sometimes several hundred females could be examined without a single female of that type appearing. However the relative number of the ab type of females appearing was a factor of considerable fluctuation and seemed to be influenced by some environmental condition. The environmental condition necessary to bring them out was not determined although close observation was made with this point in mind.

During the course of this experiment, as these females of the ab type appeared in the stocks from time to time, some of them were isolated and mated in an attempt to establish another ab strain. In some cases these strains were selected for several generations. Also some of the females of the ab type were crossed to males from the pure ab strain.

The data for these attempts are given in table 25. The strain 508 was selected for seven generations for the ab condition, but at the end of this period the strain was no nearer purity than at the beginning. In the sixth generation seven single-pair matings were made, but none of these matings showed any high percentage of abnormals. Again in strain 503, selection was carried on for three generations with no effective results. In three other cases ab females from the normal strains were mated to brothers but among the offspring there were very few if any abnormals

TABLE 25

Attempts to establish by selection a second abnormal strain.

A. Selection for ab in strain 508

Generation and mating	Female spotting				Generation and mating	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
Gen. A mating 1	124	14	3	4	Gen. F mating 2	163	11	7	12
Gen. B mating 1	219	17	13	14	Gen. F mating 3	106	9	9	10
Gen. C mating 1	217	7	6	9	Gen. F mating 4	39	9	10	4
Gen. D mating 1	252	28	24	18	Gen. F mating 5	224	24	27	23
Gen. E mating 1	160	44	22	25	Gen. F mating 6	57	6	5	10
Gen. F mating 1	61	4	6	7	Gen. F mating 7	80	10	12	14

B. Selection for ab in strain 503

Generation	Normal	Ab	Left	Right
A	289	4	3	3
B	223	11	5	3
C	147	1		1

Table 25 shows two attempts to establish a second ab strain from occasional ab females appearing in strains 508 and 503. Although strain 508 threw an unusual number of ab-appearing females, this percent could not be increased in six generations of selection. In strain 503 only a few abnormals ever appeared.

Another type of mating was made to test the genetic constitution of the ab type of females appearing in the normal strains. This was to cross the ab-appearing females to males from the pure ab strain. If these females were of the same genetic constitution as the females in the pure ab strain, a cross to the males in the ab strain should breed true for the ab condition. The results of these crosses (table 26) were, that no more abnormals appeared in the F_1 than ordinarily appeared when the males from the ab strain were crossed to normal females from the respective strains in which the ab females were found. So it seems that these constantly appearing females of the ab type in the various strains were not genotypically abnormals.

TABLE 26

Crosses of abnormal-appearing females found in wild stock to males from the pure abnormal stock.

Types of female spotting

Normal	Ab	Left	Right
109	95	20	28
60	149	19	25
67	327	23	24
68	194	16	18

The counts in table 26 give the F_1 results of crosses of the occasionally appearing ab females in wild stocks to males from the pure abnormal stock. Had these ab-appearing females been genetically pure abnormals all the offspring of the F_1 generation should have been abnormals. No higher percent of abnormals appeared in the F_1 generation than if the mating had been made to normal-spotted females from a wild strain.

Discussion of results of the analysis of the abnormal type of female spotting

From the very beginning there existed two peculiarities of the abnormal strain which made the analysis of the character both difficult and confusing. These were sexual dimorphism in the spotting of the species and the variability of the dominance or recessiveness in the F_1 generation when crossed to a normal strain.

Discussing first the origin of the ab strain,—a glance at the data might seem to indicate that the purity of the ab strain had been arrived at by the cumulative effect of selection. The data shows that it required several generations of selection to bring the strain up to a state of purity and also that a somewhat gradual change took place in the direction of selection. However it seems to the writer that the two peculiarities mentioned above offer an adequate explanation for the selection required to bring the ab strain to purity. Since the males did not show the character being selected for in the females, selection was necessarily blind as far as the paternal parent in each generation was concerned. This, together with the fact that heterozygous females might have the same appearance as pure ab females, would furnish sufficient explanation for the period of selection required to bring the ab strain to purity. The lack of success in attempting to establish pure ab strains from the occasional ab type of female appearing in normal strains indicates that the original ab individuals were probably mutations and the later-appearing were only somatic variations brought about by some environmental condition.

As to the genetic behavior of ab, early matings seemed to indicate that it had but few things in common with the behavior of a single Mendelian character. This was due largely to the lack of complete dominance or recessiveness of the ab in the F_1 generation when crossed to normal. More confusion was added when it was found that the relative number of abnormals appearing in the F_1 was influenced by the type of male spotting in the strain to which it was crossed. But after all of the data were summarized testing the effect of temperature and male spotting the following findings seem to hold true: *The ab condition in the female behaves as a Mendelian character. Its dominance or recessiveness is affected by temperature and by the type of male spotting in the normal strain to which it is crossed.* The effect of the spottedness of the males in the strain to which ab is crossed, is that the ab condition is almost entirely recessive in the F_1 generation when crossed to a normal strain in which the males are practically all of the type with a full set of six spots. The opposite holds true when ab is crossed to a normal strain where the males are of the two-spotted type. In this case ab acts more like a dominant over the normal female. High temperatures tend to reduce the number of abnormals appearing in the F_1 generation, while low temperatures increase them, but taking the effect of these factors into account the behavior of ab seemed to be that of an ordinary Mendelian character.

Summary

1. The type of female possessing the abnormal spotting probably originated as a mutation.

2. Although selection was necessary to bring the strain to purity for the abnormal type of spotting, the resulting purity was probably due only to isolation of individuals possessing the desired type of spotting, not to a cumulative effect on the strain as might be interpreted from the data. The difficulty encountered in bringing the strain to purity was due to the fact that the character was sex-limited.

3. The abnormal-spotting character acted as a Mendelian character. However the dominance or recessiveness of this character in the F_1 generation was rather variable and was found to be influenced by at least two factors.

4. One factor which influenced the behavior of the abnormal-spotting character in the F_1 generation was the strain to which the abnormal-female strain was crossed. When the strain possessing females with abnormal spotting was crossed to a strain possessing males of the six-spotted type, the former tended to act as a recessive character, but when crossed to a strain with males of the two-spotted type, it acted more like a dominant.

5. Temperature also influenced the behavior of the abnormal-spotting character in the F_1 generation. High temperatures tended to make the abnormal spotting act more like a recessive character while low temperatures cause it to act like a dominant one.

GENERAL DISCUSSION

The simplicity of a character offers no clue as to the simplicity of the manner of its inheritance. To the writer the most striking fact brought out in this study was the complexity of inheritance. A study of the spotting inheritance of a single body segment which bears only six spots might not ordinarily be expected to be very complicated. Numerous influences and interrelations of both genetic and environmental factors were found, but when carefully analyzed the inheritance was found to be Mendelian.

One of the most striking peculiarities was the almost entire independence of inheritance in the two sexes. Variations in the males of a strain did not usually influence the female spottng. The ab female and the 501 type of male were identical as far as the type of spotting was concerned. Each had the middle pair of spots missing but still there appeared to be no relation between the two when their inheritance was considered. Both males and females might possess the three pairs of spots but a different set of factors seemed to be responsible for the inheritance of the spots in each sex.

In all of the crosses between the various types of spotting studied in this paper there were no cases of complete dominance. The lack of dominance in the crossing of contrasted characters is frequently observed but its occurrence in all of the crosses in this work is worthy of note. In the case of the cross between the ab female strain and a strain of normal females, the percentage of ab females appearing in F_1 generation was dependent upon the temperature and the type of spotting of the male in the normal strain. Under certain combinations of these influencing conditions the ab condition was almost completely dominant.

Again each pair of spots seemed to be inherited independently and was acted upon differently by the same environmental factor. Low temperatures tended to intensify the outside pair of spots on the male while the same temperature tended to cause the middle pair of spots to be less distinct. Even the same pair of spots in the male was inherited differently in different strains. As simple a character as a pair of spots was dependent upon several factors.

The fact that one cannot look entirely to the germ cells for the solution of a problem in inheritance is rather strikingly brought out in this study. The importance of environmental conditions for the realization of certain characters was repeatedly seen. Temperature was shown to play an important part in making the ab condition a dominant or recessive character. The spotting in the males which seemed to be a character independent from the female spotting also influenced the dominance or recessiveness of the ab character. Again in the male spotting, certain spots which had been made to disappear by selection could be brought out again by subjection to certain temperatures. So when we consider the interrelation of different characters and the influences of environmental factors upon Mendelian characters, it is not surprising that the solution of the problem of inheritance has become more difficult than was anticipated at the time of the rediscovery of Mendel's Law.

In recent years considerable study has been devoted to the heredity of secondary sexual characters. Since the variations discussed in this paper are limited to one sex they may be considered as secondary sexual characters. It was found that the males were able to transmit variations in the female spotting which they themselves did not show. Likewise the females transmitted male variations which did not show. This ability of one sex to transmit the secondary sexual characters of the other has already been described in other insects. A similar case is that described by FOOT and STROBELL (1913) in the bug, Euschistus. They crossed two species, one of which possessed a black spot on the male. The females of both species lacked the spot. However, the female of the species of the black-spotted male transmitted the spot although she herself did not show it. Other similar cases are GEROULD'S (1911) work on *Colias philodice* and DE MEIJERE'S (1910) work on *Papilio memnon.*

Considering the findings of this study in their relation to the evolution of the species it is difficult to say just what is happening. The spotting of the female seemed to be the more stable type. In the female the full set of six spots seemed to be the normal type while in the male one pair of dorsal spots was the commoner type in nature. The frequent variations found in the male spotting would indicate that evolution was taking place in that sex. In case an evolutionary process is in progress there would be two possibilities. The process might be one of loss or acquisition of spots to the fly. In the case of the species losing spots, all of the males would have once possessed six spots but have been the first of the two sexes to be acted upon by the process. On the other hand if the species be acquiring spots the action of the process upon the male has been retarded, the female having already changed from a two-spotted type to a six-spotted one. These are only suggestions of what might possibly be taking place, as there is no evidence from which we can draw any dependable conclusions.

GENERAL SUMMARY

1. This paper is a discussion of spotting inheritance in the fruit fly, *Drosophila busckii.* The spotting of the fifth abdominal segment is the subject of discussion.

2. There is sexual dimorphism in the spotting of this segment, the normal female having three pairs of spots on this segment and the male having one, two, or three pairs of spots on it.

3. An unusual type of female was found (probably originated as a mutant in the writer's stocks) which had one of the three pairs of spots missing. This was found to bear no relation to a similar type of spotting in the male; in fact the various types of spotting found in the two sexes were inherited independently.

4. The mutant in female spotting required considerable selection to bring the strain to purity, but the effect of selection was probably only to isolate individuals homozygous for the character.

5. Extensive selections were made for the type of male having one

pair of spots and the type having three pairs of spots. The strains in which selection was made for the two types of males were of a common parentage. By selection strains were produced which had a high percentage of the desired type but neither was ever brought to absolute purity for the type sought.

6. The behavior of the mutant in female spotting in the F_1 generation was affected by the strain to which it was crossed. If crossed to a strain possessing a high percentage of six-spotted males, it acted as a recessive character but if crossed to a strain with a high percentage of two-spotted males, it acted more like a dominant.

7. High temperatures caused the mutated type of female spotting to act as a recessive character while low temperatures made it act as a dominant.

8. In the male spotting, high temperatures intensified one pair of spots while the same temperatures seemed to reduce another pair of spots on the same segment.

9. In the male spotting, when a strain selected for two-spotted males was crossed to a strain selected for six-spotted males neither type of spotting was dominant in the F_1 generation.

10. There was a difference in amenability to selection of different strains taken from nature; but when two strains, each selected for six-spotted males but of different origin, were crossed, the results of the cross indicated that their spottedness was due to the same factors.

11. An unusual type of male spotting was found in which the spots of one pair were very large. A study of this character indicated that sex-linked inheritance was involved in its genetic behavior.

Acknowledgments are due Professor FERNANDUS PAYNE for many helpful suggestions and criticisms during the course of this study. Also I am indebted to my wife, ELMIRA SHIERLING WARREN, for much assistace both in the breeding work and preparation of material for this paper.

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GENETICS, JANUARY 1920

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OUR FRONTISPIECE

Joseph Gottlieb Koelreuter, M.D., the first scientific hybridizer of plants, was born at Sulz am Nekar, Bavaria, April 27, 1733, and died at Karlsruhe, Baden, November 12, 1806. His first hybrid resulted from a cross made in 1760 between *Nicotiana rustica* and *N. paniculata*, and the F_1 hybrids were grown in 1761.

The portrait which is here reproduced is a photographic copy of the only known portrait of Koelreuter. At latest available report (now somewhat out of date) the original was in the possession of W. Koelreuter, Hornberg, Baden, a grandson of this great pioneer geneticist. The portrait has been engraved a number of times. In the original the bust is set in an oval, but a photographic copy of this has been retouched and worked over into the present oblong form by J. Marion Shull, of the U. S. Bureau of Plant Industry, Washington, D. C.

We are enabled to present this portrait to our subscribers through a gift generously given by Professor Oakes Ames, of Harvard University, well known for his numerous papers on the Orchidaceae.

VOLUME 5 NUMBER 2

GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS BEARING ON HEREDITY AND VARIATION

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GENETICS

A Periodical Record of Investigations Bearing on
Heredity and Variation

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STUDIES IN MILK SECRETION. V. ON THE VARIATIONS AND CORRELATIONS OF MILK SECRETION WITH AGE[1]

JOHN W. GOWEN
Maine Agricultural Experiment Station, Orono, Maine

[Received September 8, 1919]

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INTRODUCTION

A large body of exact scientific data on milk production has been accumulated since the time of the establishment of the ADVANCED REGISTRY SYSTEM of the HOLSTEIN-FRIESIAN ASSOCIATION OF AMERICA and its later adoption by the Associations of the other breeds of dairy cattle. These completed records are unique in several ways, chief among which

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is the fact that the records of the cows included among them must meet a certain standard of performance. This standard for entry has the effect of cutting out certain records of cows of the given breed. Thus the JERSEY REGISTRY OF MERIT (THE AMERICAN JERSEY CATTLE CLUB 1906) says that, "if the test is commenced the day the cow is two years old, or previous to that day, she must produce within one year from the date the test begins, 250.5 pounds of butter-fat. For each day the cow is over two years at the beginning of her year's test, the amount of butter-fat she must produce in the year is fixed by adding 0.1 (one-tenth) of a pound for each such day to the 250.5 pounds required when two years old. This ratio of increase applies until the cow is five years old at the beginning of her test, when the required amount will have reached 360 pounds, which will be the amount of butter-fat required of all cows five years old or over. These standards are based upon one complete year's record from the time of beginning, regardless of any time which may be lost by being dry or calving during the period." Three facts are obviously true of the JERSEY REGISTRY OF MERIT cows as compared with a true sample taken at random of the milking cows of the Jersey breed; (1) the cows making up the REGISTRY OF MERIT are a selected sample; (2) the scale of the selection is linear having its lower limit 250.5 pounds of butter-fat production at 2 years and its upper limit 360 pounds at 5 years and over; (3) this requirement means that for each day of age at test that frequency distributions of years production are cut off perpendicularly at the requirement and only those animals making greater yields than this are allowed to be entered into the REGISTRY OF MERIT. The data taken from this REGISTRY OF MERIT are not the true data for the Jersey breed and conclusions based on it cannot be considered as applying to the breed as a whole or to the general problems of milk secretion.

MATERIAL AND METHODS

To supply this need of exact data on the Jersey breed as a whole the MAINE AGRICULTURAL EXPERIMENT STATION has obtained the recorded data on one of the largest pure-bred Jersey herds known. The data are exceptional in the following ways: (1) The records extend back to the year 1897 when the herd was organized; (2) the animals are practically all straight Island stock: (3) they have been under the oversight and direction of one manager since 1901; (4) exact records are kept of the milk production, butter fat and butter-fat percent; (5) many of the individual animals have records for several different lactations. The elimination of variation of the milk production of cows or groups of cows caused by changes of any one or more of these five factors is important for the analysis of the causative mechanism of milk and butter-fat production. It is obvious that these records are free from such variables. They constitute a homogeneous group of data representing the Island Jersey under constant conditions of management and climate.

Such an accumulation of exact statistical data on the cows of the Jersey breed for problems of so much interest, both biologically and economically, warrants the application of adequate biometrical methods in their analysis. Such analysis is now well recognized by most investigators as of the utmost importance to our understanding of the fundamental principles of physiology which underlie the process of milk secretion itself.

The general problems attacked are those of the individual variation between the lactating functions of different dairy cows at a given age. What is the true type of the frequency distributions of milk production? What relation exists between the mean productions of the successive ages in a true random sample of the Jersey breed? An understanding of these and similar questions is necessary to the fuller utilization of the data found in the herd book of the registry association for as previously pointed out the requirement for entrance acts as a true truncating agent to the frequency distribution of a given age. Without a knowledge of the type of these frequencies no true correction factors may be determined to properly allow for the increase in age of the cows either for direct comparison of yields or for inheritance studies.

The biometrical methods used are in general those of any adequate analytical treatment of a quantitative subject. In each case reference to the source of the original publication where the methods may be found are given where the formula is first used. In each case the standard deviations have SHEPPARD's correction applied to them as a glance at the data shows that high contact is present in the distributions.

The data used for study are all from normal, healthy cows. Two diseases have been present in the herd, tuberculosis and abortion. The tuberculous animals were all eliminated early in the herd's history by the use of the tuberculin test. All records from animals which were proven to be tubercular or which aborted were not used. Records from animals normally healthy but sick during a given lactation were not used. All of the cows have been kept in climatic conditions similar to those of western Virginia.

A word as to the method of keeping the data and its transfer to this

Station is perhaps necessary. All records are made at the time of milking on the daily milk sheets for the given cow which are kept in the barn. The milking takes place twice a day, the records are for night and morning. The weekly production taken from these sheets is transferred to the herd ledger by a trained bookkeeper. The total production for a given month is found together with the yearly production by adding the weekly totals. All records are recorded to pounds and tenths. The cows are tested bi-monthly by the Babcock test and the percentage of butter-fat is recorded beside its corresponding monthly milk yield. All weighings and readings are recorded immediately after they are made so there is little chance of inaccuracy. From these records the author has extracted 1741 complete 8-months records of healthy cows for milk production. Of these 1741, 1713 have records for the butter-fat percent. The weighted monthly averages of the bi-monthly test have been used to obtain the weighted 8-months average for the 8-months lactation period chosen for study.

VARIATION OF JERSEY MILK PRODUCTION WITH AGE AT COMMENCEMENT OF TEST

The tabulation of these records in complete eight-months records has been done by the author. The choice of a proper interval of grouping for age was decided upon as one year, that is, grouping all of those records commenced while the cows were 2 years, no months and no days, to 3 years, no months and no days, and so on to include up to 10 years, no months and no days. The group 10 years-and-above has been included in one group as the numbers were quite small in the older age classes. The absolute and percentage frequencies determined for the different age classes are shown in table 1.

Table 1 together with figure 1 makes clear the following points in regard to frequency distributions of milk production.

(1) The frequency polygons show milk production to be quite symmetrical.

(2) The range of variation is quite considerable, extending from one cow which produced under 1500 pounds for the eight-months period to one which produced over 10,000 pounds.

(3) The polygons change their shape with increase in age at the commencement of the test, those of the early ages being higher at the mode and less scattered than those of the later years.

(4) There appears to be only one mode in all of the distributions. From this it seems probable that the polygons belong to the unimodal

Table I

Frequency distributions of variations for the eight-months lactation records of a homogeneous herd of Jersey cows at different ages. Absolute and percentage frequencies.

Pounds of milk produced	Age, 2 yrs., 0 mos., to 3 yrs., 0 mos.		Age, 3 yrs., 0 mos., to 4 yrs., 0 mos.		Age, 4 yrs., 0 mos., to 5 yrs., 0 mos.		Age, 5 yrs., 0 mos., to 6 yrs., 0 mos.		Age, 6 yrs., 0 mos., to 7 yrs., 0 mos.	
	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency
1000 to 1500	1	.3								
1500 to 2000										
2000 to 2500	5	1.6	3	1.2						
2500 to 3000	23	7.6	7	2.8	2	.8			2	1.0
3000 to 3500	60	19.7	27	10.7	15	6.1	12	5.4	6	3.1
3500 to 4000	58	19.1	31	12.3	26	10.5	26	11.7	16	8.3
4000 to 4500	67	22.0	47	18.7	44	17.8	28	12.6	23	12.0
4500 to 5000	54	17.8	49	19.4	44	17.8	33	14.8	23	12.0
5000 to 5500	23	7.6	36	14.3	49	19.8	40	17.9	29	15.1
5500 to 6000	11	3.6	21	8.3	28	11.3	25	11.2	24	12.5
6000 to 6500	2	.7	15	6.0	15	6.1	19	8.5	28	14.6
6500 to 7000			6	2.4	11	4.5	16	7.2	13	6.8
7000 to 7500			7	2.8	6	2.4	13	5.8	13	6.8
7500 to 8000			2	.8	5	2.0	5	2.2	7	3.6
8000 to 8500			1	.4	1	.4	4	1.8	4	2.1
8500 to 9000					1	.4	1	.4	2	1.0
9000 to 9500									1	.5
9500 to 10000									1	.5
10000 to 10500							1	.4		
Total	304	100.0	252	100.1	247	99.9	223	99.9	192	99.9

TABLE 1 (continued)

Frequency distributions of variations for the eight-months lactation record of a homogeneous herd of Jersey cows at different ages. Absolute and percentage frequencies.

Pounds of milk produced	Age, 7 yrs., 0 mos., to 8 yrs., 0 mos.		Age, 8 yrs., 0 mos., to 9 yrs., 0 mos.		Age, 9 yrs., 0 mos., to 10 yrs., 0 mos.		Age, 10 yrs., 0 mos., to above		Total population	
	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency
1000 to 1500									1	.1
1500 to 2000										
2000 to 2500	2	1.2			2	2.4	1	.9	13	.7
2500 to 3000	1	.6	1	.8	6	7.1	3	2.8	50	2.9
3000 to 3500	7	4.1	9	7.1	5	5.9	10	9.3	166	9.5
3500 to 4000	16	9.4	15	11.8	6	7.1	8	7.5	212	12.2
4000 to 4500	24	14.1	15	11.8	11	12.9	18	16.8	279	16.0
4500 to 5000	20	11.8	13	10.2	15	17.6	21	19.6	274	15.7
5000 to 5500	28	16.5	27	21.3	15	17.6	19	17.8	266	15.3
5500 to 6000	21	12.4	15	11.8	5	5.9	12	11.2	162	9.3
6000 to 6500	20	11.8	13	10.2	8	9.4	7	6.5	127	7.3
6500 to 7000	14	8.2	9	7.1	8	9.4	5	4.7	82	4.7
7000 to 7500	10	5.9	5	3.9	2	2.4	2	1.9	58	3.3
7500 to 8000	3	1.8	2	1.6	1	1.2	1	.9	26	1.5
8000 to 8500	3	1.8	1	.8	1	1.2			15	.9
8500 to 9000	1	.6							3	.2
9000 to 9500									2	.1
9500 to 10000			1	.8					2	.1
10000 to 10500			1	.8					3	.2
Total	170	100.2	127	100.0	85	100.1	107	99.9	1741	100.0

type although this point cannot be accurately determined, as the histograms are quite flat-topped.

The accuracy of these conclusions is further brought out by a study of the chief physical constants of the distributions. Table 2 gives the four constants, mean, standard deviation, coefficient of variation, and skewness, together with their probable errors for each frequency of age.

TABLE 2

Constants of variation of milk production for the successive ages at test in Jersey milk.
(Eight-months lactation period.)

Age at test	Mean	Standard deviation	Coefficient of variation	Skewness
2 yrs., 0 mos., to 3 yrs., 0 mos.	4032.9 ± 31.7	818.9 ± 22.4	20.304 ± .577	
3 yrs., 0 mos., to 4 yrs., 0 mos.	4686.5 ± 46.8	1101.5 ± 33.1	23.503 ± .712	+0.2642 ± .0548
4 yrs., 0 mos., to 5 yrs., 0 mos.	4992.9 ± 46.3	1079.4 ± 33.0	21.619 ± .684	+0.3532 ± .0635
5 yrs., 0 mos., to 6 yrs., 0 mos.	5281.4 ± 57.0	1262.8 ± 40.3	23.911 ± .801	+0.3894 ± .0714
6 yrs., 0 mos., to 7 yrs., 0 mos.	5536.5 ± 64.5	1325.3 ± 45.6	23.938 ± .865	+0.2586 ± .0628
7 yrs., 0 mos., to 8 yrs., 0 mos.	5314.7 ± 64.9	1255.0 ± 45.9	23.613 ± .907	
8 yrs., 0 mos., to 9 yrs., 0 mos.	5226.4 ± 77.9	1302.0 ± 55.1	24.912 ± 1.111	+0.3141 ± .1173
9 yrs., 0 mos., to 10 yrs., 0 mos.	4938.2 ± 95.3	1302.6 ± 67.4	26.377 ± 1.453	
10 yrs., 0 mos., and above	4838.8 ± 70.3	1077.3 ± 49.7	22.264 ± 1.028	
Total population	4887.6 ± 20.2	1249.7 ± 14.3	25.569 ± .310	+0.3150 ± .0222

It seems best to postpone the complete discussion of the facts brought out in this table until other data to be presented are available. A few general points of especial interest may be mentioned, however. The mean milk production for the eight-months period for those cows which are between 2 and 3 years of age is 4032.9 pounds. From this point the milk production rises rapidly at first, then more slowly to a maximum at about 7 years. From this maximum the decline in milk production is less rapid toward the higher ages.

The difference between the production of 2 years and 6 months and the maximum is significant, as the difference is about 20 times its probable error (1503.6±71.9). The difference between the maximum production and that for the 10 years and above is also significant, as the difference is more than 6 times the probable error (697.7±95.40). This rise and fall of milk production with age has already been shown, by the work of this laboratory (PEARL 1914), to conform to that group of curves described by a logarithmic function.

The standard deviation varies between 818.9 pounds at the age of 2 to 3 years to 1325.3 pounds at the age of 6 to 7 years. The rise of the standard deviation is more direct than is the rise of the mean curve. From the maximum the curve remains parallel with the base line for some years when it again drops to a lower deviation at the highest age at test. This rise and fall is significant as judged by the probable errors of the difference (PEARL 1909). The difference of the maximum standard deviation at 6 to 7 years from that at 2 to 3 years is 506.4 pounds of milk where the probable error is 50.8 pounds, or the difference is practically 10 times the probable error and the probability that this difference did not come from random sampling is considerably greater than 1,000,000,000 to 1 (ELDERTON 1901, PEARSON 1900). The difference between the maximum standard deviation and that at 10 years and above is 248.0 pounds and the probable error is 67.5 pounds or 3.68 times the probable error and the probability that this difference did not come from random sampling is 75.7 to 1. The conclusion seems justified from the above facts that the standard deviation varies with age at test in such a way that the absolute amount of the standard deviation is least at the early age, increases rapidly to a maximum at about 5 years and 6 months, remains at this maximum for about four years then falls again toward the later ages.

The coefficient of variation for the milk production of the successive ages are all high as compared with those already known for other similar data. This large size in the coefficients is brought out by table 3.

TABLE 3

Comparison data for coefficients of variation of amount of secretion.

Organism	Secretion	Coefficient of variation	Source of data
Domestic fowl (Barred Plymouth Rock)	Annual egg production	34.21 ± .37	(PEARL 1909)
Cattle, British Holstein	Gallons of milk	25.72 ± .372*	(GAVIN 1912)
Cattle, Ayrshire	Gallons of milk	24.187 ± .504†	(VIGOR 1913)
Cattle, Jersey	Pounds of milk	25.569 ± .310	This paper

* Probable error calculated by author from totals given in GAVIN's paper.

† Calculated by the author from the means and standard deviation as given by VIGOR (1913).

Of the substances studied annual egg production is the most variable. The coefficients of variation for milk production correspond remarkably well considering the diversity of the sources from which they are taken. They all go to show that milk production varies around a mean of 25, or about 9 percent less variable than egg production. This difference is significant. The mechanism of the secretion of the sum of the egg parts to form the egg is shown to be more variable than that for the secretion of the milk parts to form the milk. Taking this reasoning back to its ultimate source, such a significant difference shows that the secretory cells of the mammary glands work with greater precision than do the cells of the oviduct. Such a difference in the precision of action of the two sets of cells would seem to indicate a greater approach to perfection, in a mechanical sense, in the cells of the udder than is true of the cells of the oviduct.

The skewness of the two sets of data furnish another interesting contrast. The skewness for annual egg production for Barred Plymouth Rock hens is —0.205 whereas the skewness for Jersey milk production is +0.315. Not only is the sign different but the actual amount is different. This cannot be explained on the basis of any selection for high producers that may have taken place, as both sets of data are about equally subject to such selection. The data for the successive age groups all go to show that where the distributions for milk production are skew they are all plus. This lends further strength to the belief that the distribution for the yearly production of the two sets of glands is skew in opposite directions. One thing is common in the two cases, the skewness in each is small in amount. This is of especial importance as it shows an approach to the value where the typical Gaussian curve of error may describe these functions.

ANALYTICAL CONSTANTS DESCRIBING THE VARIATION OF MILK PRODUCTION AT DIFFERENT AGES

The general physical constants of milk production having been considered, the further analysis of its problems requires a knowledge of the types of frequency distributions which go to make up the lactation record of a cow during her life time. In the solution of this kind of data comes the answer to such problems as, does the type of milk-production distribution of cows milking at two years old differ from that of cows milking at three years old. This question is of great importance in considering the milking records of advanced-registry animals where it is necessary to form an opinion of the capacities of a breed from the milking abilities of a selected sample where the selection is not at random but removes those cows producing under a certain amount.

This feature of the problem is seen in the work of REITZ (1909) on the inheritance of butter-fat production in Holstein Friesian cattle.

The data for this problem came from the advanced registry of Holstein Friesian cattle. Since as a requirement to entry in this registry the cows must produce more than a certain amount of butter-fat, the correlation from such data measuring the strength of inheritance are subject to a double selection. RIETZ in correcting for this selection uses the method devised by PEARSON to determine the whole of a normal curve when a portion of it is known. The accuracy of the corrections depend then on the curves for milk production and for butter-fat percentage being normal.

The data on which this paper is based are excellent for an answer to these problems as to the types of the production curves for cows at different ages, as they represent a herd of pure-bred Jersey cows, accurately tested and recorded, constituting as near a random sample of the breed under uniform conditions as could well be found. The constants describing these distributions are given in Table 4.

TABLE 4

Analytical constants of variation in milk production for the successive ages at test for Jersey milk. (Lactation period, eight months.)

Constants	2 yrs., 0 mos., to 3 yrs., 0 mos.	3 yrs., 0 mos., to 4 yrs., 0 mos.	4 yrs., 0 mos., to 5 yrs., 0 mos.
Mean	4032.89 ± 31.67	4686.51 ± 46.80	4992.91 ± 46.33
Standard deviation	818.85 ± 22.40	1101.47 ± 33.09	1079.40 ± 32.96
Coefficient of variation	20.304 ± .577	23.503 ± .742	21.619 ± .684
μ_2	+ 2.6821	+ 4.8529	+ 4.6604
μ_3	+ 0.2894	+ 5.0413	+ 6.2588
μ_4	+ 19.9393	+ 74.5962	+ 73.6275
β_1	0.0043 ± .0023	0.2224 ± .1003	0.3870 ± .1391
β_2	2.7718 ± .1362	3.1675 ± .3244	3.3900 ± .3811
$\beta_2 - 3$	— 0.2282	+ 0.1765	+ 0.3900
κ_1	— 0.4694 ± .2719	— 0.3322 ± .4483	— 0.3811 ± .4631
κ_2	— 0.0070 ± .0070	— 0.5303 ± 1.2280	—0.8361 ± 2.9443
Skewness		+ 0.2642 ± .0548	+ 0.3532 ± .0635
Modal divergence		+ 291.06 ± 73.48	+ 381.219 ± 75.52
Mode		4395.45	4611.70
Type of curve	II	I	I
+ end of curve	8069.15	17695.15	18896.33
— end of curve	3.36	1314.12	2351.50
y_0	71.7407	45.9314	47.7073

TABLE 4 (continued)

Analytical constants of variation in milk production for the successive ages at test for Jersey milk. (Lactation period, eight months.)

Constants	5 yrs., 0 mos., to 6 yrs., 0 mos.	6 yrs., 0 mos., to 7 yrs., 0 mos.	7 yrs., 0 mos., to 8 yrs., 0 mos.
Mean	5281.39 ± 57.04	5536.46 ± 64.52	5314.71 ± 64.92
Standard deviation	1262.84 ± 40.34	1325.32 ± 45.62	1254.98 ± 45.91
Coefficient of variation	23.911 ± .801	23.938 ± .865	23.613 ± .907
μ_2	+ 6.3791	+ 7.0259	+ 6.2999
μ_3	+ 10.1997	+ 7.5096	+ 3.0843
μ_4	+ 134.5412	+ 144.0314	+ 105.1169
β_1	0.4008 ± .1436	0.1626 ± .0833	0.0380 ± .0300
β_2	3.3063 ± .3745	2.9178 ± .2565	2.6485 ± .1655
$\beta_2 - 3$	+ 0.3063	— 0.0822	— 0.3515
κ_1	— 0.5898 ± .4413	— 0.6523 ± .3714	— 0.8171 ± .3021
κ_2	— 0.5619 ± .5240	— 0.1952 ± .1928	— 0.0354 ± .0347
Skewness	+ 0.3894 ± .0714	+ 0.2586 ± .0628	
Modal divergence	+ 491.70 ± 103.81	+ 342.77 ± 107.10	
Mode	4789.69	5193.69	
Type of curve	I	I	II
+ end of curve	16682.37	13960.06	10186.64
— end of curve	2430.70	1961.51	442.78
y_0	35.9420	28.3749	25.6501

TABLE 4 (continued)

Analytical constants of variation in milk production for the successive ages at test for Jersey milk. (Lactation period, eight months.)

Constants	8 yrs., 0 mos., to 9 yrs., 0 mos.	9 yrs., 0 mos., to 10 yrs., 0 mos.	10 yrs., 0 mos., and above
Mean	5226.38 ± 77.92	4938.24 ± 95.30	4838.79 ± 70.25
Standard deviation	1301.98 ± 55.10	1302.56 ± 67.38	1077.28 ± 49.67
Coefficient of variation	24.912 ±1.111	26.377 ± 1.453	22.264 ± 1.028
μ_2	+ 6.7806	+ 6.7867	+ 4.6421
μ_3	+ 13.8706	+ 1.1758	+ 1.4117
μ_4	+ 201.7722	+ 118.7966	+ 60.7500
β_1	0.6171 ±.4429	0.0044 ± .0497	0.0199 ± .0150
β_2	4.3886 ± 1.7775	2.5792 ± .2166	2.8191 ± .2517
$\beta_2 - 3$	+ 1.3883	— 0.4208	— 0.1809
κ_1	+ 0.9258 ± 2.4700	— 0.8548 ± .3509	— 0.4216 ± .4975
κ_2	+ 0.5794 ± 1.4184	— 0.0039 ± .0051	— 0.0357 ± .0424
Skewness	+ 0.3141 ± .1173		
Modal divergence	+ 408.93 ± 156.62		
Mode	4817.45		
Type of curve	IV	II	II
+ end of curve	+ ∞	9498.86	10852.89
— end of curve	— ∞	377.61	1175.32
y_0	.0014	12.2016	19.3754

TABLE 4 (continued)

Analytical constants of variation in milk production for the successive ages at test for Jersey milk. (Lactation period, eight months.)

Constants	Total population
Mean	4887.56 ± 20.21
Standard deviation	1249.71 ± 14.28
Coefficient of variation	25.569 ± .310
μ_2	+ 6.2471
μ_3	+ 9.6623
μ_4	+ 138. 3606
β_1	0.3829 ± .0613
β_2	3.5454 ± .1979
$\beta_2 - 3$	+ 0.5454
κ_1	— 0.0581 ± .2582
κ_2	— 5.4182 ± *
Skewness	+ 0.3150 ± .0222
Modal divergence	+ 393.65 ± 29.91
Mode	4493.91
Type of curve	I
+ end of curve	95879.24
— end of curve	1029.4
y_0	288.674

* Probable error very large.

Attention may be directed first to the moments of the individual distributions of milk production for the groups of the successive yearly ages. The third moment is consistently plus and on the whole rather small. The moments lead to values of β_1 which are rather small. In no case is the value so great as three times its probable error. β_2 is likewise only slightly different from the value 3 demanded by the normal curve. In no case is this difference as great as three times its probable error. Out of the nine age groups into which the data have been divided β_2—3 is plus four times and minus five times. No consistency is shown in the sign and it seems safe to say its change of direction comes from random sampling. The sign of κ_1 is plus once and minus eight times; its value in no case exceeds three times its probable error. The sign of κ_2 is of course, governed by that of κ_1. The absolute value varies between 0.0039 and 0.8361. The values of the probable errors are all large in comparison with the values of κ_2.

These facts all lead to the conclusion that no great confidence can be placed in either the sign or the absolute magnitude of the criterion for the frequency type. Such big probable errors of κ_1 and κ_2 suggest that all of the curves are near the critical limit of more than one type of frequency curve. The problem now becomes: what is the chance that a frequency distribution, other than that indicated by the criteria may be obtained to fit the data equally well. It is necessary therefore to turn to the probable errors of the frequency type itself to determine this question. The tables and diagrams necessary for the speedy determination of this have been calculated by RHIND (1909). Comparison of the values of Σ_1 Σ_2 with diagram for the frequency type contained in this paper together with the other necessary calculations shows the types indicated in table 4 should give a good fit.

The distributions for the successive ages are shown to be equally types I and II with one which is type IV. Five of the curves are skew and four symmetrical; eight are limited in range in both directions and one is unlimited in range. The equations to these curves are as follows.

Age at test 2 years to 3 years:

$$y = 71.7407 \left(1 - \frac{x^2}{16,291,354}\right)^{10.648}.$$

Age at test 3 years to 4 years:

$$y = 45.9314 \left(1 + \frac{x}{3081.32}\right)^{6.2322} \left(1 - \frac{x}{13299.70}\right)^{26.8997}$$

Age at test 4 years to 5 years:

$$y = 47.7073 \left(1 + \frac{x}{2260.20}\right)^{4.0347} \left(1 - \frac{x}{14284.63}\right)^{25.4999}$$

Age at test 5 years to 6 years:

$$y = 35.9420 \left(1 + \frac{x}{2358.99}\right)^{2.8776} \left(1 - \frac{x}{11892.68}\right)^{14.5074}$$

Age at test 6 years to 7 years:

$$y = 28.3749 \left(1 + \frac{x}{3232.17}\right)^{3.8103} \left(1 - \frac{x}{8766.38}\right)^{10.3344}$$

Age at test 7 years to 8 years:

$$y = 25.6501 \left(1 - \frac{x^2}{23,735,702}\right)^{6.0352}$$

Age at test 8 years to 9 years:

$$y = .0014 \left(1 + \frac{x^2}{12,094,088}\right)^{-9.9809} e^{21.0802 \tan^{-1} \frac{x}{3477.66}}$$

Age at test 9 years to 10 years:

$$y = 12.2016 \left(1 - \frac{x^2}{20{,}799{,}307}\right)^{4.6294}$$

Age at test 10 years and above:

$$y = 19.3754 \left(1 - \frac{x^2}{36{,}169{,}519}\right)^{14.0831}$$

Total population:

$$y = 288.674 \left(1 + \frac{x}{3464.52}\right)^{8.086} \left(1 - \frac{x}{91385.33}\right)^{213.294}$$

The histograms and the fitted curves for the milk production of the individual ages are shown in figure 1. All of the areas have been reduced to a percentage base so that the ordinates and areas of each distribution could be compared directly.

It is clear to the eye that on the whole the graduation of the data by the fitted curves is rather good. Measured mathematically by the test of goodness of fit (ELDERTON 1901; PEARSON 1900) of the theoretical curve to the raw data the value of P ranges between 0.5864 and 0.9942 as shown by table 5.

TABLE 5

Values of P for the fit of the theoretical curves to the observational curves for milk production of Jersey cattle.

Age at test	P
2 years to 3 years	0.6680
3 years to 4 years	0.8453
4 years to 5 years	0.9114
5 years to 6 years	0.9424
6 years to 7 years	0.9942
7 years to 8 years	0.9145
8 years to 9 years	0.5864
9 years to 10 years	0.6873
10 years and above	0.9602

This table brings out the excellence of the graduation of the data by the frequency curves.

As previously stated eight of the nine curves are of limited range in both directions and one of unlimited range in either direction. The limits of these ranges are of especial interest since they indicate the highest and lowest milk production expected for a given age. It is evi-

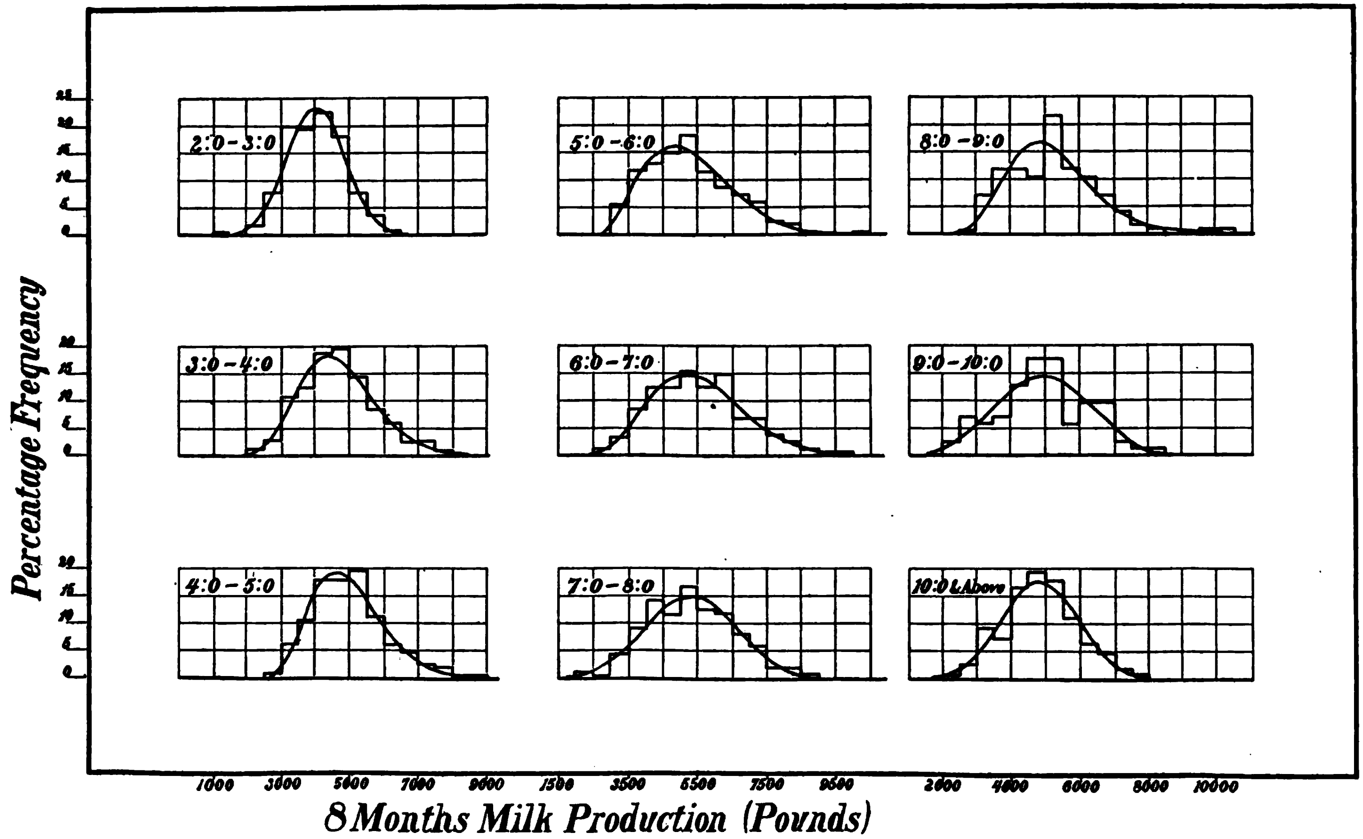

FIGURE 1.—Histograms and fitted curves showing the variation of milk production for each year of age. The area of each curve is reduced to unity so that any distribution of a given age may be compared with ony other at another age. The relative increase in the height of the model ordinates at the lower and higher ages of lactation as compared with the ages 6 to 10 years is quite evident. The fitted curves are all PEARSON type curves the constants of which are seen in table 4.

dent that in the symmetrical curves (type II) the plus end of the range is not as high as in the type I curves, in fact, the end of the range of high milk producers is rather underestimated than overestimated. The type I curves estimate the highest expected milk production at what we might consider a fair estimate. Thus, Jersey cows 3 years to 4 years old would not be expected to produce more than 17,695 pounds of milk in 8 months. This is borne out by the actual experience of the breed. The figure 10,186 for the 8-months production in the 7-year to 8-year class is low, however, compared with the production of certain REGISTRY-OF-MERIT cows; e.g., Passport is known to have produced 19,694 pounds in one year. Such a difference in the plus end of the range would seem to indicate a real difference of this group of Jerseys from those of the advanced registry group in the direction of an extended range for the REGISTRY-OF-MERIT animals.

The minus end of the range is equally interesting. The type II curves again do not give as good an estimate as the type I. They are in most cases too low. The low end of the production scale for this group of cattle is on the whole pretty well estimated, however. For the data themselves we have one observation as low as 1250-pound group. The estimate for this particular range is on the whole rather low (3.36 pounds of milk). In any case the author has in his note-book a record of milk production for one year of an Aberdeen Angus cow at 6 years 6 months of only 244.7 pounds which is not so far different from the above low-range estimate. Such records as these show us that the low margin of production is not on the whole overestimated. In all of this work it must also be remembered that the limited- and unlimited-range curves have only a small frequency at these points. Thus the fact that we have one curve whose range is not limited should not trouble us, as the frequencies of this range beyond the customary limits, become very small indeed.

All of the curves are of the mesokurtic type as the values of β_2—3 are small and in each case less than three times the probable error of β_2. Furthermore the sign of the kurtosis is plus in four cases and minus in five showing more clearly if necessary the mesokurtic nature of all of the distributions.

The changes in the position of the modal ordinate with increasing age generates a curve rising almost linearly to its high point at a production of 5314 pounds for 7 years and 6 months. From this high point it falls toward a lower production for the maximum frequency of the older cows.

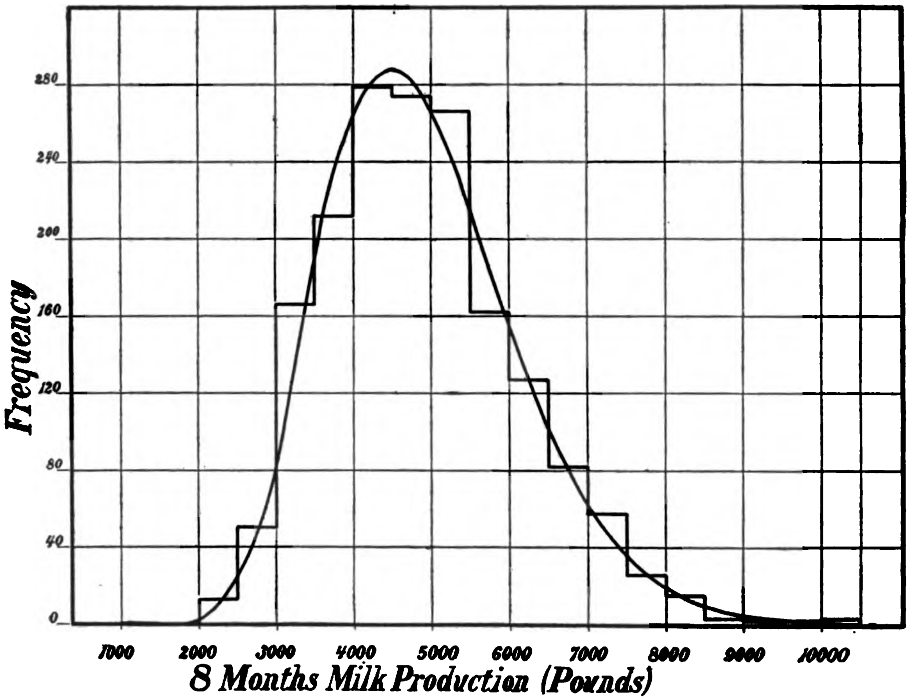

FIGURE 2.—Histogram and fitted curve showing the variation of milk production that may be expected to occur in a pure-bred Jersey herd without regard to age. It is evident that by and large the curve strikes through the observation well. The distribution of milk production is seen in general to be quite skew.

The variation of the total population of Jersey milk production is valuable both for itself and for comparison with other breeds. Table 4 gives the necessary constants for the calculation of the frequency curve suitable to fit the data. Figure 2 shows these data graphically.

The theoretical curve for these data is type I as the values of β_1 and β_2 are such as to lead to a κ_2 of —5.4182 calling for a type I curve. The curve is significantly skew in the plus direction. The range of this curve much more than covers the actual observations and goes a good deal beyond the probable value of the milk production of any cow in describing the highest milk production expected. This is no doubt due to the fact that toward the plus end of the range the observed frequency of high-producing cows trails off slowly. The minus end of the range is well described.

As shown by the above data the assumption previously indicated in the study of milk inheritance by RIETZ is open to the criticism that the distributions of milk production at a given age are not normal curves

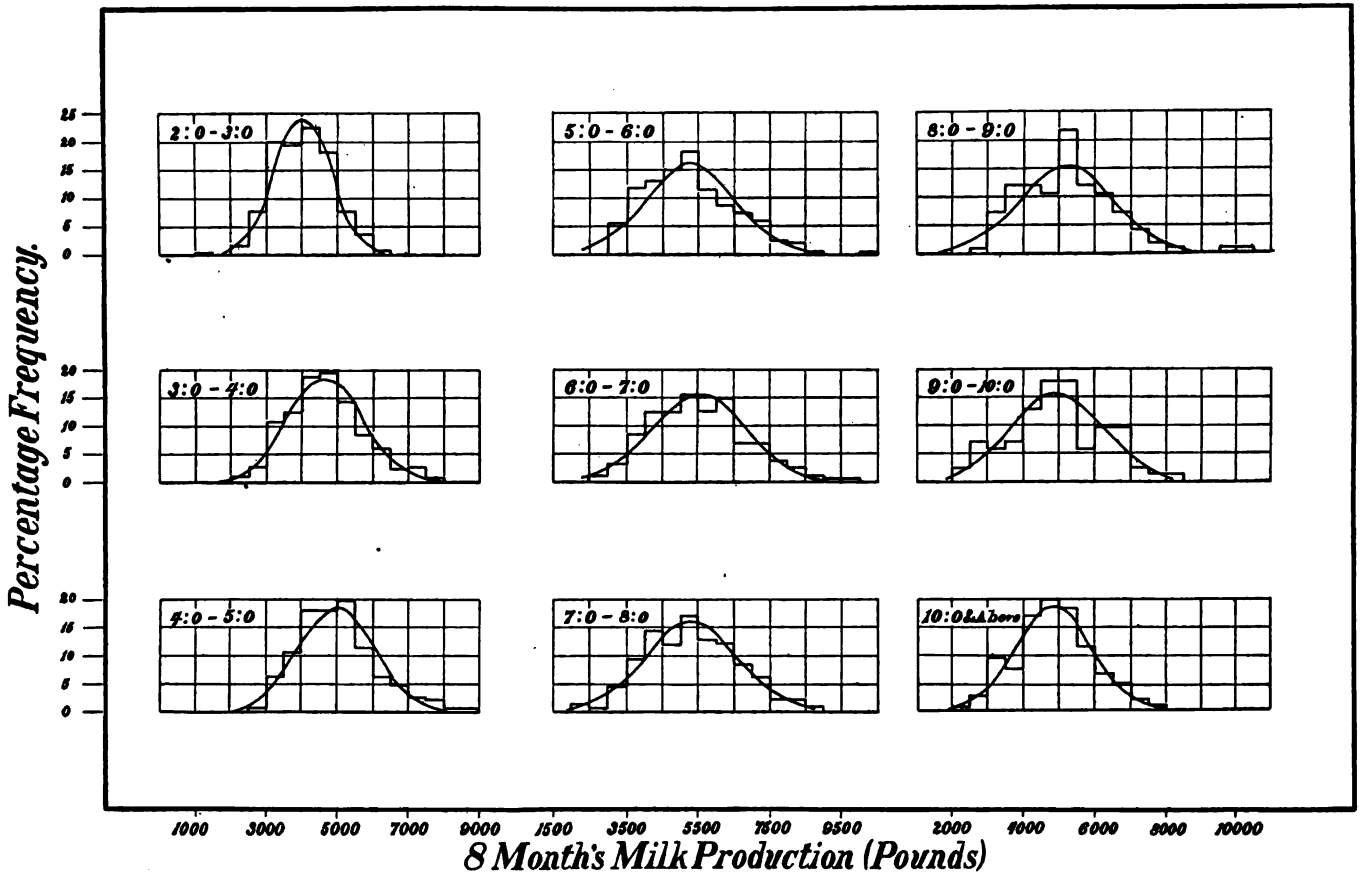

FIGURE 3.—Histograms and fitted curves showing the variat'on of milk production of each year of age. The fitted curves in this case are all normal curves calculated from table 4. The histograms are the same as those of figure 1. Each distribution is drawn on a basis of area equal to one, so that each curve is directly comparable with others. It is evident that the fit as a whole is not as good as in figure 1, especially at the ends of the range.

but belong in general to the type I and type II curves. The problem becomes that of how great an error is introduced by regarding the data as described by normal curves. Normal curves have been fitted to the above data to make the comparison. These curves together with their corresponding histograms are shown in figure 3.

In comparison with figure 1 the normal curves are seen in general to describe the tail frequencies poorly. The P for the fit of these curves are shown in table 6.

TABLE 6

Values of P for the fit of the theoretical normal curves to the observational curves for milk production of Jersey cattle.

Age at test	P
2 years to 3 years	0.4335
3 years to 4 years	0.4130
4 years to 5 years	0.0943
5 years to 6 years	0.7442
6 years to 7 years	0.7450
7 years to 8 years	0.8532
8 years to 9 years	0.5517
9 years to 10 years	0.5083
10 years and above	0.9333

These data (table 6) show that normal curves do not describe milk production at different ages as well as do the proper type curve. The difference in the fit of the type curves over the normal curve comes especially in the description of the tails and the skewness of these distributions. The fit of the normal curves is not bad, however, and in all probability no serious error would be made in the use of them for the determination of the corrections to be applied to the correlations from double-selected data as was done by RIETZ.

THE CORRELATION OF JERSEY 8-MONTHS MILK PRODUCTION WITH THE AGE AT TEST

Common knowledge among dairymen is that milk production and age at the commencement of the lactation are correlated in such a way that advancing age means increased production. The opinion is general that this increase is a linear one. This opinion has been shown to be erroneous by previous work of this laboratory (PEARL 1914) on the statistics of the 7-day records of American Jersey cattle found in "Jersey sires with their tested daughters," published by the AMERICAN JERSEY

Cattle Club. In this work seven-day milk production is shown to be a logarithmic curve and not a straight line.

It remains to be shown what is the curve describing a true random sample of the Jersey breed for a longer milking period. Data of this sort is important for several reasons, chief among which, both practically and theoretically, is the necessity of having suitable correction factors for age to allow comparison of milk records at different periods in the lives of different cows. Toward the solution of this problem the following facts are necessary: What correlation exists between age and milk production? Is this correlation sufficient so that it must be taken into account in considering records of different cows at different ages? What is the equation of the regression line between these two variables? Table 7 furnishes the data necessary for this study.

The correlation and its accompanying constants for these two variables are shown in table 8.

Table 8

Constants measuring the association between amount of eight-months milk produced and age at test of Jersey cows.

r	η	$\eta - r$	$\eta^2 - r^2$
0.2596 ± .0151	0.4283 ± .0132	0.1689 ± .0201	0.1161 ± .0108

This table makes clear several facts concerning the influence of age on milk production. The correlation of +0.2596±.0151 shows that age at test and milk production are significantly correlated variables. Taken in conjunction with the correlation ratio it shows clearly that age of the cow at commencement of test must be considered in comparing the records of different cows if the conclusion from the comparison is to be valid. The value of the correlation ratio +0.4283 ±.0132 is considerably higher than the correlation coefficient. This difference is shown to be highly significant by the value of $\eta - r = 0.1689$ ±.0201. It is altogether probable therefore that the regression of age on milk production is a skew regression. This is shown to be a fact by the constant to measure such skewness. $\eta^2 - r^2$, 0.1161±.0108, is about 11 times its probable error. The regression is therefore known to be skew. Since this is true the correlation ratio is a better measure of the true correlation than is the correlation coefficient. The relation of age at test is then doubly significant in any comparison of the records of two cows. The regression having been shown to be skew it becomes necessary to deal with it separately.

Corry cattle. (Lactation period, eight months.)

	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	10500-11000	Total
1:6- 2:0									34
2:0- 2:6									255
2:6- 3:0									49
3:0- 3:6	2								156
3:6- 4:0	5	2	1						96
4:0- 4:6	1	2		1					139
4:6- 5:0	5	3	1						108
5:0- 5:6	9	2	1	1					108
5:6- 6:0	4	3	3				1		115
6:0- 6:6	6	3	3			1			92
6:6- 7:0	7	4	1		2		1		100
7:0- 7:6	5	2							83
7:6- 8:0	5	1	3	1					87
8:0- 8:6	2	1	1				1		71
8:6- 9:0	3	1				1			56
9:0- 9:6	1								43
9:6-10:0	1	1	1						42
10:0-10:6									26
10:6-11:0	1	1							24
11:0-11:6	1								26
11:6-12:0									5
12:0-12:6									9
12:6-13:0									7
13:0-13:6									5
13:6-14:0									2
14:0-14:6									2
14:6-15:0									0
15:0-15:6									1
15:6-16:6									
Total	58	26	15	3	2	2	3		1741

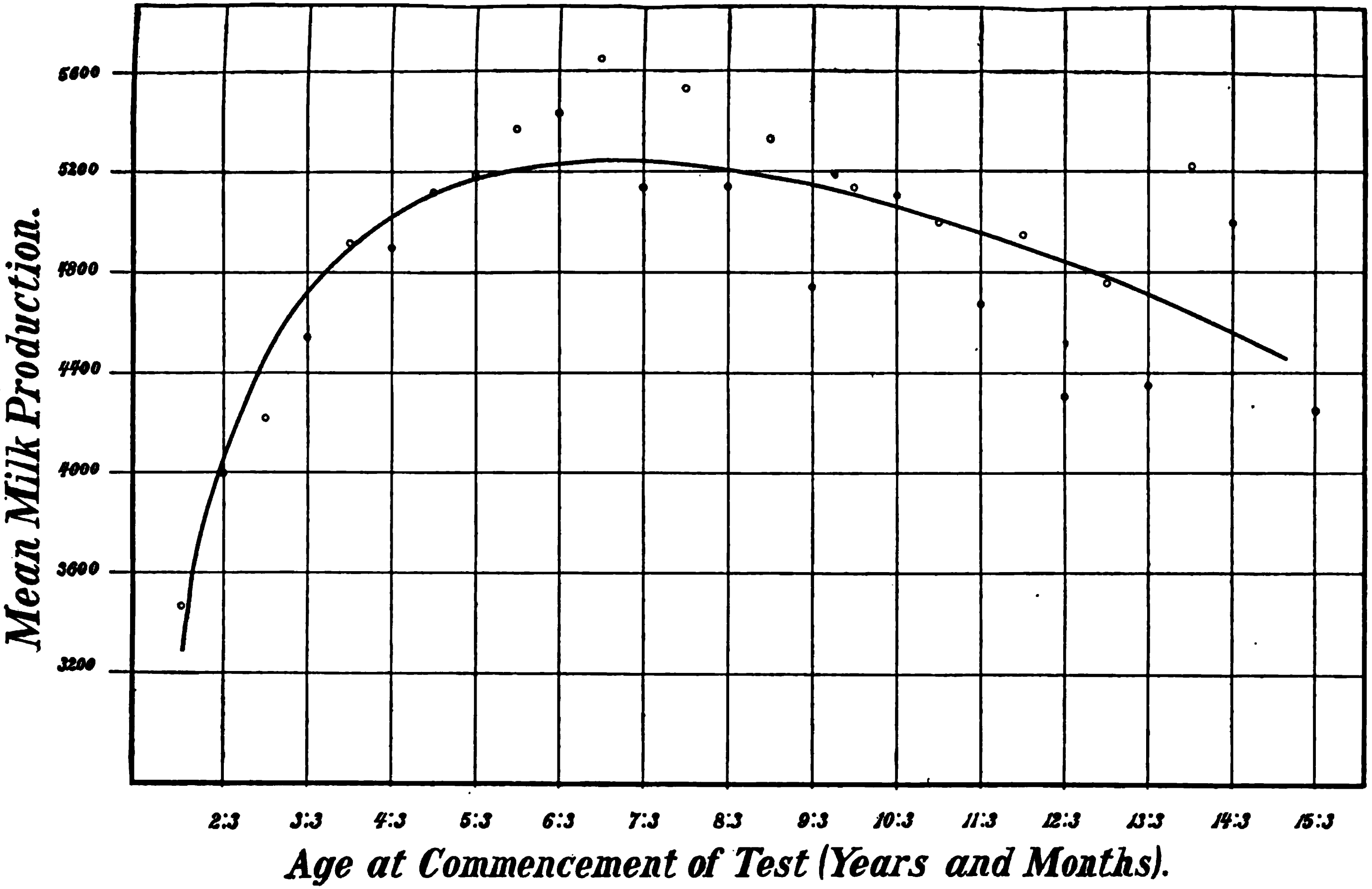

FIGURE 4.—Diagram showing the observed means and theoretical means for Jersey eight-months milk production at any given age. The observed means are calculated from table 7. The curve as a whole is evidently of the logarithmic type described well by the theoretical curve the constants of which are given below. The observations are represented as the small circles.

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TYPES OF THE REGRESSION OF MILK PRODUCTION ON AGE OF JERSEY CATTLE

The means for each array of age of table 6 have been calculated. These means are indicated by points within circles in figure 4.

Examination of the observational curve in figure 4 shows that it takes the general form of a logarithmic curve, agreeing in this respect with the other data from this laboratory (PEARL 1914). From these means the theoretical curve conforming to the general logarithmic type has been calculated by the method of least squares. The equation to this curve is

$$y = 3387.912 - 99.883x - .487x^2 + 2896.219 \log x$$

where x is taken in six-months intervals from an origin at 1 year and 3 months.

The observations at the higher ages vary a good deal as they are based on small numbers. The theoretical curve strikes through them quite accurately when the unevenness of the observed curve is considered. When we calculate the χ^2 by the method of SLUTSKY (1914) we find that 5 observations contribute a sum of 28.80 to the total of 45.41. These observations are at ages 2 years 9 months, 3 years 3 months, 6 years 9 months, 7 years 9 months and 9 years 3 months. If we measure the fit by the total χ^2, 45.41, it is poor. Considering the above-mentioned five observations in connection with the other observations it is seen that two of them are plus and three are minus quantities. Not only that but they come at places in the curve so that they could practically counteract each other if the first smoothed curve were used as the observational. It seems altogether reasonable therefore to consider the fit of this curve measured by a χ^2 somewhat more than 17.00 or what would correspond to the P of a very good fit.

The equation of the curve has many practical uses aside from its interest in a scientific sense. By its use the records of cows at different ages may be brought to the same basis for comparison whether it be for milk-inheritance studies, analysis of judging experiments or the like. The time of the theoretical maximum of milk production may be easily calculated from it by differentiation. This maximum is shown to be 7 years 2.4 months, a figure considerably above the age customarily called mature form. Further the curve shows that the method used in advanced-registry work of determining the amount a Jersey cow should produce for the REGISTER OF MERIT is fallacious in that it is a linear method and does not recognize this logarithmic nature of milk production. In a previous paper (GOWEN 1919) the average fat percent of

Jersey cows is given as 5.12. Assuming this figure and dividing the pounds of butter-fat by it gives us the average requirement for milk production in one year. Supposing that ¾ of the year's records is made in the first 8 months of lactation (a figure reasonably close to the expected (PEARL 1915)) the required production is found to be 3600 pounds at 2 years and 5200 pounds at 5 years. From figure 3 it is evident therefore that if a cow is to be pushed for the advanced registry the best time to get her in, is to have her calve at about 3 years 3 months of age.

Causally considered the logarithmic nature of milk production is of a good deal of interest. The work of a number of students of growth, beginning with MINOT'S (1891) notable studies on rabbits and guinea-pigs has shown that the phenomena of growth are also a logarithmic function of age. This law appears of wide general application as the work of LEWENZ and PEARSON (1904) have shown that it holds for growth in children; DONALDSON (1908, 1909, 1910, 1911), HATAI (1911) and JACKSON (1913) have shown it to be of general application to the growth of certain organs in the white rat; PEARL (1907) and PEARL and SURFACE (1915) have shown it to be true for Ceratophyllum and maize. It seems, therefore, altogether likely that the mammary glands of the cow also follow this rule. Should this prove true the increase of milk production with age seems of much significance in paralleling these growth phenomena. This paralleling of the two functions would in fact seem to indicate a causal relation between the two in that the increase in milk production may depend chiefly on the increase in actual mass of the mammary gland due to growth of this organ and not due to any relative increase in the ability of the cells to secrete more milk.

THE NATURE OF THE CURVE OF THE STANDARD DEVIATION OF MILK PRODUCTION

The curve found by plotting the standard deviations of 8-months milk production for each six months age intervals is shown in figure 4. The circles represent the actual standard deviations of the age arrays calculated from table 7. A glance at these suffices to show that the curve is not the customary linear one. It has rather the appearance of a parabola or logarithmic curve. The actual choice of the type of curve describing these data, other things being equal, depends on the fit of the theoretical curve to the observational data. Accordingly two curves were tried, a cubic parabola having the form $y = a + bx + cx^2 + dx^3$, and a logarithmic curve $y = a + bx + cx^2 + d \log x$. These two curves were chosen as they had the same number of constants. The cubic fitted to the data by the method of MINER (1917) gave the equation to the data as

$$y = 399.46 + 188.82\,x - 11.664\,x^2 + 0.1919\,x^3.$$

The logarithmic curve was

$$y = 497.08 + 4.025\,x - 1.689\,x^2 + 909.13 \log x$$

The fit of these curves to the observational data was then compared by calculating for each the mean error and the root mean-square error. These values will of course be very large from the evident unevenness of the observation curve, they will however serve as a good comparative measure. The mean error for the parabola is 99.6, the root mean square error 132.0; for the logarithmic curve the mean error is 102.3 and the root mean square error 133.5. From this comparison the cubic parabola fits the data most accurately. Figure 5 shows the observation and fitted cubic. The cubic parabola is seen by the figure to strike through the observation very well.

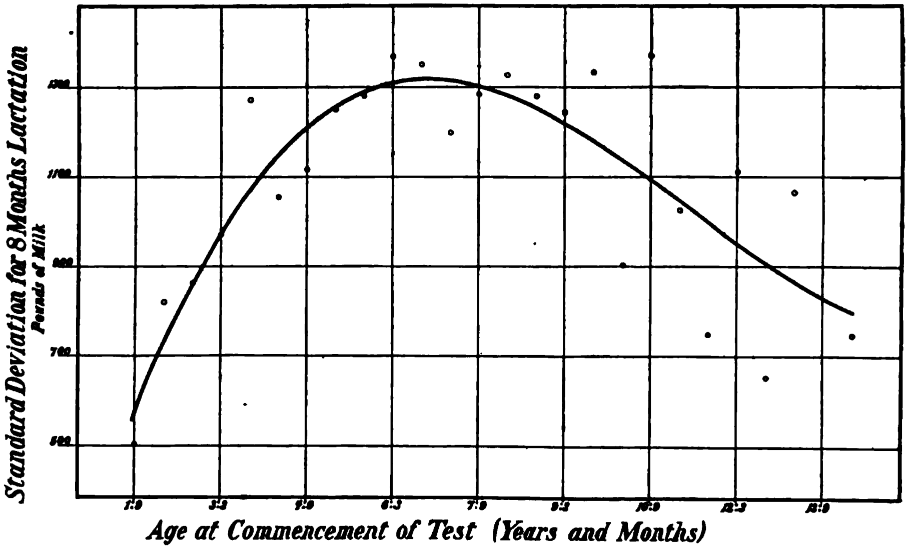

FIGURE 5.—Observational and theoretical curve for the standard deviations of milk production for the successive ages at which the lactation commenced. The theoretical curve is the cubic parabola given above. Considering the large variation of the observed standard deviations the cubic strikes through the observations well.

If we define with PEARSON (1905) the scedastic curve as the curve formed by the ratios of standard deviations of the x arrays of β divided by the standard deviation of β plotted to x, then it is evident that the standard-deviation lines of milk production show that the value of η is will form, when divided by the standard deviation of milk production at all ages, a curve similar in shape to the curve plotted in figure 5. Milk production with age is therefore heteroscedastic. As the change in the scedastic curve follows a regular system the curve is nomic.

The facts brought forth in the discussion of the regression and standard-deviation lines of milk production show that the value of η is nearer the value of the true correlation of milk production with age than is the value of r. It therefore strengthens more strongly if need be the argument of age correction in all milk-production studies involving comparisons of milk production at different times in a cow's life.

THE CORRELATION BETWEEN THE MILK YIELD OF ONE LACTATION AND THAT OF SUCCEEDING LACTATIONS

Mean milk production of cows retained long or short periods in the herd

Up to this point the discussion has dealt entirely with the influence of age on amount of milk produced in a given lactation and the variability of the milk produced during it. In the present section the phase of the problem dealing with one lactation in its relation to another will be considered.

The functioning of the mammary glands may be considered dependent upon three main factors, which, taken in order of their natural sequence are: heredity; development through feeding, etc. (environmental circumstances), of these organs up to their commencing to secrete; and lastly environmental factors in their widest sense acting during the months when the gland is active. It is reasonably clear that on our ability to distinguish the relative influence of these three basic variables depends many of the common *a priori* dairy practices as well as furnishing a solid foundation for the analysis of the causal mechanism of milk production itself. The analysis is a complex one and needs to be attacked by many channels. The present investigation was undertaken in the hope that by an analysis of the intra-individual variation of milk secretion from lactation to lactation some light would be given on the relative merits of these three variables. The homogeneous nature of the material is such, however, that the investigation necessarily deals chiefly with the first of these variables.

Little work on milk secretion has been done that approaches the problem from this viewpoint. Of the available data those on the English herds analyzed by GAVIN (1913) are undoubtedly the best. This investigation on a mixed herd of British Holsteins and grade Short Horns furnishes data of value for the records of the first lactation in comparison with the yields of subsequent lactations. In all of this work the measure of the lactation used is what he designates as the "revised maximum," this term being defined as the maximum day yield of the lactation which is three times reached or exceeded. These results are considered largely for their strictly practical bearing. They are of little use to the American farmer in that he is accustomed to deal with records over a certain limit of time and not maximum productions. These facts make it evident that no repetition in either data or aim of the investigation occurs in these studies. The constants derived by GAVIN will be of a good deal of interest for comparison with those presented here as together they show the range of variation to be expected under the different conditions of England and the United States, a mixed herd and a pure-bred herd, and a difference in the measure of the lactating capacity from lactation to lactation.

The preliminary steps should include a study of the population of each frequency which is to be compared. The data for this comparison are taken from the correlation tables beginning with number 9 and ending with 40.

TABLE 9

Correlation surface for amount of eight-months milk production at two years old and amount of eight-months milk production at three years old for pure-bred Jersey cows.

	3 years old													
2 years old	2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	Total
2000-2500			1											1
2500-3000			1	5	5									11
3000-3500	1	1	8	5	11	5	1	1						33
3500-4000			3	6	11	11	5	3						39
4000-4500			2	2	4	11	7	4	3	1	1			35
4500-5000		2	2	2	4	8	6	6	7	2				39
5000-5500						2	5	2	2	1	3	1	1	17
5500-6000			1		1	2			1	1	1			7
6000-6500											1	1		2
Total	1	3	18	20	36	39	24	16	13	5	6	2	1	184

TABLE 10

Correlation surface for amount of eight-months milk production at two years old and amount of eight-months milk production at four years old for pure-bred Jersey cows.

4 years old

2 years old	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	Total
2500-3000	1	4	4		2								11
3000-3500	5	6	10	2	5	1							29
3500-4000	12	3	7	7	8	3		1					41
4000-4500	2	2	5	6	9	4	7	3	1	1	1		41
4500-5000	1		5	6	8	7	1	2	3				33
5000-5500			1	2	2	1	1	3	2	1			13
5500-6000					1	1	2	1				1	6
Total	21	15	32	23	35	17	11	10	6	2	1	1	174

The tabled constants are presented in the following order, (1) the means of each of the compared populations (table 11), (2) the standard deviations of each of the compared populations (table 20), and the coefficients of variation of each of the compared populations (table 25). The vertical columns of each table present the means, standard deviations, etc., of the age indicated when correlated with each of the lactation records made at the age on the left of the table. Thus, the means of the 8-months' milk production of the two-year group that is correlated with the three-year group, is 4141.30 pounds of milk. The value of the mean production in this column should of course be the same if the population of milk production of the two-year group is the same for the cows kept to their three-year-old lactation as it is for the cows kept to their seven-year-old lactation. Table 11 presents the data for the comparisons of these mean milk productions.

TABLE 11

Mean 8-months milk production of a given age when correlated with other given ages.

Age	2-3	3-4	4-5	5-6
2 to 3		4796.20 ± 55.15	4962.64 ± 59.63	5542.86 ± 74.47
3 to 4	4141.30 ± 40.12		5031.05 ± 60.19	5344.41 ± 80.52
4 to 5	4091.95 ± 37.66	4642.16 ± 57.56		5190.55 ± 67.40
5 to 6	4178.57 ± 44.62	4739.51 ± 04.10	5033.22 ± 59.05	
6 to 7	4192.62 ± 48.71	4819.57 ± 72.78	5141.67 ± 67.93	5401.64 ± 76.40
7 to 8	4156.59 ± 58.81	4867.35 ± 77.53	5143.81 ± 65.17	5315.89 ± 65.87
8 to 10	4061.93 ± 48.09	4698.11 ± 75.14	5067.07 ± 62.08	5298.28 ± 69.64
10 and older	4162.50 ± 66.27	5171.05 ± 199.66	4994.90 ± 103.53	5468.18 ± 65.79

TABLE 11 (continued)

Mean 8-months milk production of a given age when correlated with other given ages.

Age	6-7	7-8	8-10	10 and older
2 to 3	5754.10 ± 88.77	5464.29 ± 86.47	5094.04 ± 81.11	4925.00 ± 119.59
3 to 4	5710.87 ± 89.16	5459.18 ± 87.31	5099.06 ± 91.12	4907.89 ± 144.32
4 to 5	5612.50 ± 104.63	5435.84 ± 80.10	5152.44 ± 78.20	4760.20 ± 101.39
5 to 6	5631.15 ± 83.22	5521.32 ± 73.79	5208.62 ± 75.90	4868.18 ± 108.99
6 to 7		5334.68 ± 78.04	5189.19 ± 71.69	4914.06 ± 84.65
7 to 8	5399.19 ± 115.94		5081.13 ± 74.40	4831.08 ± 85.76
8 to 10	5371.62 ± 74.90	5220.20 ± 63.66		4903.23 ± 69.14
10 and older	5382.81 ± 61.45	5195.94 ± 83.20	5185.48 ± 69.65	

The data presented in this table are graphically represented in figure 6. In this figure the mean 8-months milk production is given at the left. The line at the top of the figure represents the mean milk production of the two-year-olds that had lactations at the other ages shown on the lower margin of the figure. The next line down represents the 3-year mean production when compared with the other ages, etc. The ages with which these are compared are shown in the bottom margin of the figure. The lines generated by the mean productions of each of these age groups should, of course, be parallel with the base of the figure within the limits of random sampling if the population of 8-months milk production for the given age is the same irrespective of the age at which the subsequent lactation was made.

The curve for the mean eight-months milk production of the two-year-old group (the top curve of figure 6) is as close to a straight line parallel with the base of the figure as could well be expected. At no place is there more than 65-pound divergence from such a line. This divergence is seen in table 11 to be only slightly over the probable error of the mean production.

The curve for the three-year-old group is more variable than the two-year group due no doubt to the numbers on which it is based being less. The curve on the whole has a slight tendency upward as we progress from left to right. It is doubtful if this general trend is significant in comparison with the probable errors of the constants. Taken at its face value, however, it would indicate that the cows with a lactation record at 10 years of age would have had in general a slightly better record at three years old than would the general three-year-old group. That is, the cows of the three-year-old group were culled slightly so that the cows kept for milk production were somewhat better cows than the average run of three-year-olds.

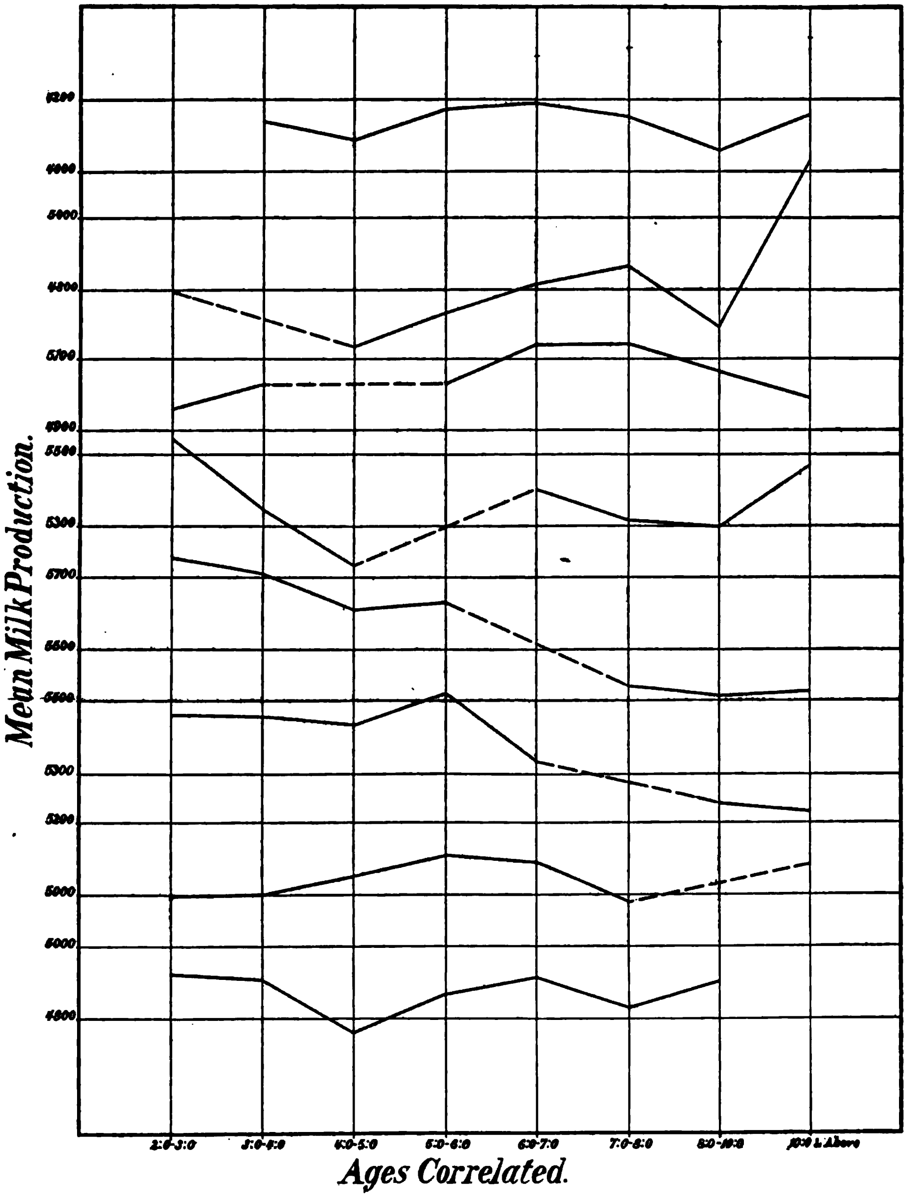

FIGURE 6.—Curves showing the mean eight-months milk production of a given age at each successive age with which the given age is correlated. The ordinates are the pounds of milk produced and the abscissae are the ages of the groups with which the given age is correlated. Proceeding from top to bottom of the figure the first line represents the mean milk production of the 2-year to 3-year group; the second the 3-year to 4-year group; the third the 4-year to 5-year; the fourth the 5-year to 6-year; the fifth the 6-year to 7-year; the sixth the 7-year to 8-year; the seventh the 8-year to 10-year, and the last the 10-year-and-older group. The lines obviously are quite irregular but in general do not diverge greatly from a line parallel with the base of the figure.

TABLE 12

Correlation surface for amount of eight-months milk production at two years old and amount of eight-months milk production at five years old for pure-bred Jersey cows.

5 years old

2 years old	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	Total
2000-2500	1															1
2500-3000		1	2			2										5
3000-3500	1	5	6	9	2	2	1	1								27
3500-4000	2	2	4	3	6	3		1		2						23
4000-4500	1	2		8	9	4	5	3	1	1	2					36
4500-5000	1			3	5	3	4	6	4	2					1	29
5000-5500	1				1	1	3	2	4							12
5500-6000					1		1	1	1	1						5
6000-6500									1		1					2
Total	7	10	12	23	24	15	14	14	11	6	3	0	0	0	1	140

TABLE 13

Correlation surface for amount of eight-months milk production at two years old and amount of eight-months milk production at six years old for pure-bred Jersey cows.

6 years old

2 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	Total
2000-2500		1															1
2500-3000		1	2	1		1				1							6
3000-3500		1	5	5	5	1	2	2	1								22
3500-4000		1	1	4	2	1	3	3	2								17
4000-4500		1	1		1	7	7	5	2	5		3					32
4500-5000					4	5	4	5	1	4	2			1			26
5000-5500	1			1			2	1	3	1	1	1	1		1		13
5500-6000								1		1						1	3
6000-6500										1	1						2
Total	1	5	9	11	12	15	18	17	9	13	4	4	1	1	1	1	122

Table 14

Correlation surface for amount of eight-months milk production at two years old and amount of eight-months milk production at seven years old for pure-bred Jersey cows.

2 years old	7 years old: 2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	Total
2000-2500			1												1
2500-3000			.	1	1	2									4
3000-3500	1		1	5	2	4	4			2					19
3500-4000				1	1	3	3	2	1	2					13
4000-4500			1	1	1	4	9	3	4	2		1			26
4500-5000					1			3	3	5			1	1	14
5000-5500							2	2	1		3				8
5500-6000							2		1				1		4
6000-6500										1	1				2
Total	1	0	3	8	6	13	20	10	10	12	4	1	2	1	91

Table 15

Correlation surface for amount of eight-months milk production at two years old and amount of eight-months milk production at eight and nine years old for pure-bred Jersey cows.

2 years old	8 and 9 years old: 2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	10000-10500	Total
2000-2500		1		1													2
2500-3000				4	1		2										7
3000-3500	1	1	1	6	4		1	2		1							17
3500-4000		1	1	1	2	3	6	3	4								21
4000-4500			1	4	5	3	6	5	2	4	2						32
4500-5000	1					7	3		3	3	2						19
5000-5500							4	2	1	1						1	9
5500-6000									1		1						2
Total	2	3	3	16	12	13	22	12	11	9	5	0	0	0	0	1	100

The four-year- and five-year-old records are practically linear and parallel with the base. No selection has taken place in these cows to weed out the poor milkers.

The six- and seven-year curves both appear linear but have a downward tendency which looks significant when compared with its probable errors. Thus in the six-year group the difference is 371.3±107.9 or 3.4 times its probable error. Furthermore the drop in the value of the milk production of the six-year group is quite sudden, occurring with those cows which lactate in their seventh year and older. Selection can-

TABLE 16

Correlation surface for amount of eight-months milk production at two years old and amount of eight-months milk production at ten years and older for pure-bred Jersey cows.

10 years old and older

2 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	Total
2500-3000	1				1							2
3000-3500		1		2	1	1						5
3500-4000		1		1		1						3
4000-4500		2	2	5	5	3	3		1		1	22
4500-5000						3		2				5
5000-5500									2			2
5500-6000									1			1
Total	1	4	2	8	7	8	3	2	4	0	1	40

TABLE 17

Correlation surface for amount of eight-months milk production at three years old and amount of eight-months milk production at four years old for pure-bred Jersey cows.

4 years old

3 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	Total
2000-2500		1												1
2500-3000	1		1	1			1							4
3000-3500		3	4	3	2	1								13
3500-4000		2	3	9	5	4	1							24
4000-4500		4	2	7	9	10	1	1		1			1	36
4500-5000			1	6	4	7	5	2	2					27
5000-5500		1		1	5	6	2	2	2					19
5500-6000					3	2	2	2	1	1				11
6000-6500					1	2	2		2	1				8
6500-7000								3	1	1				5
7000-7500				1					1		1	1		4
7500-8000											1			1
Total	1	11	11	28	29	32	14	10	9	4	2	1	1	153

not account for this drop for it goes directly opposite to the direction in which selection would be made unless the highly unlikely assumption is made that the cows with high six-year-old records die before their seventh lactation. This assumption is known not to be true as the records of death are accurately kept. The history of the herd is accurately known and while perhaps a half a dozen old cows purchased at its beginning their production was fair and they were of ages 6 and older with no centering on any age. No other possible reason for the drop is known to the author. The reason for this drop consequently remains obscure.

The curves for the eight-and-nine-year and the ten-and-above groups all appear linear and parallel with the base, indicating that no selection had been practiced in choosing these animals.

To sum up the results of this study, no clear evidence of selection of animals to be kept as further milk producers is evidenced by the mean milk production of any age in comparison with the other lactations that the cow was known to be milking.

TABLE 18

Correlation surface for amount of eight-months milk production at three years old and amount of eight-months milk production at five years old for pure-bred Jersey cows.

5 years old

3 years old	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	Total
2000-2500	1															1
2500-3000	2				1	1			1							5
3000-3500	3	3	5	3	2	1										17
3500-4000	1	4	2	6	1		1			1						16
4000-4500		5	4	6	5	1		1								22
4500-5000	1	1	4	5	5	6	3		1	1						27
5000-5500	1		1	2	6	1	2	4	3						1	21
5500-6000	1			1	1	4	1	1	2	1						12
6000-6500				1	1	3		5	2	1						13
6500-7000						1	1		1							3
7000-7500							1	1	2		1					5
7500-8000											1					1
Total	10	13	16	24	22	18	9	12	12	4	2	0	0	0	1	143

TABLE 19

Correlation surface for amount of eight-months milk production at three years old and amount of eight-months milk production at six years old for pure-bred Jersey cows.

6 years old

3 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	Total
2000-2500						1											1
2500-3000	1				1		1										3
3000-3500		2	2	5	1	3	1										14
3500-4000		1	4	1	2	2			1	1							12
4000-4500			2	1	3	3	3	1								1	14
4500-5000				2	1	2	3	7	3	2		1					21
5000-5500	1				2	4	5	4	1	3	2						22
5500-6000				1	1		2	2		1				1	1		9
6000-6500					1	2	1	2	2			2					10
6500-7000						1				1	1						3
7000-7500							1		1	1	2						5
7500-8000											1						1
Total	2	3	8	10	12	18	17	16	8	9	6	3	0	1	1	1	115

The standard deviations of milk production as influenced by the length of time the cow is retained in the herd

A similar analysis for the effect of selection of cows at a given age on the basis of their milk production to be retained as milkers in after years is given in table 20. The arrangement of the data is entirely similar to that of table 11. The vertical columns give the standard deviations (without SHEPPARD's correction) for the age of the cows indicated; the values are, from top to bottom, those of the given year when the same-age cows also have other records at the years indicated on the

TABLE 20

Standard deviations of milk production of a given year when correlated with other given years.

Age	2 years	3 years	4 years	5 years
2 to 3		1109.14 ± 39.00	1166.17 ± 42.17	1306.56 ± 52.67
3 to 4	806.99 ± 28.37		1103.81 ± 42.56	1427.69 ± 56.94
4 to 5	736.58 ± 26.63	1055.55 ± 40.70		1195.11 ± 47.66
5 to 6	782.79 ± 31.55	1136.60 ± 45.33	1047.07 ± 41.76	
6 to 7	797.53 ± 34.44	1157.00 ± 51.45	1103.37 ± 48.04	1251.05 ± 54.02
7 to 8	831.76 ± 41.59	1137.94 ± 54.83	1027.18 ± 46.09	1109.12 ± 46.57
8 to 10	744.36 ± 34.00	1147.04 ± 53.13	1020.66 ± 43.89	1243.36 ± 49.25
10 and older	621.36 ± 46.86	1290.28 ± 141.18	1074.39 ± 73.20	1174.59 ± 75.54

TABLE 20 (continued)

Standard deviations of milk production of a given year when correlated with other given years.

Age	6 years	7 years	8 and 9 years	10 and older
2 to 3	1453.51 ± 62.76	1222.82 ± 61.14	1255.63 ± 81.12	1121.10 ± 84.54
3 to 4	1417.51 ± 63.04	1281.58 ± 61.75	1390.96 ± 64.43	932.63 ± 102.05
4 to 5	1699.38 ± 73.99	1262.45 ± 56.65	1285.72 ± 55.29	1052.16 ± 71.68
5 to 6	1362.64 ± 58.84	1235.78 ± 51.89	1355.07 ± 53.67	1198.34 ± 77.07
6 to 7		1288.48 ± 55.19	1293.65 ± 50.69	1004.11 ± 59.87
7 to 8	1274.72 ± 54.60		1355.50 ± 52.61	1093.68 ± 60.63
8 to 10	1236.58 ± 48.47	1159.80 ± 45.01		1141.55 ± 48.89
10 and older	1108.36 ± 66.08	1061.13 ± 58.83	1149.98 ± 49.25	

left margin of the table. The data for the calculation of these constants are taken from correlation tables 9 to 40.

These data are graphically represented in figure 7, the values of the standard deviations being represented as ordinates and the ages at which the cows had another lactation as the abscissae. The curves progressing from top to bottom of the figure are for the lactation at the 2, 3, 4, 5, 6, 7, 8-and-9 and 10-and-older groups. The lines generated by those curves should, of course, be parallel with the base if there has been no selection practiced. If selection has been at any given place it should be expected to curtail the low-producing cows and thus reduce the standard deviation of the population as a whole.

TABLE 21

Correlation surface for amount of eight-months milk production at three years old and amount of eight-months milk production at seven years old for pure-bred Jersey cows.

	7 years old														
3 years old	2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	Total
2500-3000		1													1
3000-3500	1		2	1	1	2	3								10
3500-4000			2	3	4	2	1	1		1				1	15
4000-4500				3	1	3	1	2	1	1	1				13
4500-5000					2	4	4	1	4	3			1		19
5000-5500					2	2	2	2	2	2	2		1		15
5500-6000							1		2	1		1			5
6000-6500							4	1	3	3					11
6500-7000							1	2		1					4
7000-7500									1		3				4
7500-8000										1					1
Total	1	1	4	7	10	13	17	9	13	13	6	1	2	1	98

TABLE 22

Correlation surface for amount of eight-months milk production at three years old and amount of eight-months milk production at eight and nine years old for pure-bred Jersey cows.

3 years old	8 and 9 years old: 2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	Total
2500-3000		1	1			2			1	1								6
3000-3500	1	2	3	3		1	2	1	2									15
3500-4000		1	2	3	3	2	2											13
4000-4500				3	1	1	2	1	1		1							10
4500-5000			1	2	2	5	5	2	1									18
5000-5500				1	2	2	6	2	5	1		1						20
5500-6000					2	1			2								1	6
6000-6500					2	1	2	2	2	1	2							12
6500-7000							1			1	2							4
7000-7500												1				1		2
Total	1	4	7	12	12	15	20	8	14	4	5	2	0	0	0	1	1	106

The curves for the two, three, four, five, seven, eight-and-nine and ten-and-older age groups are obviously linear and follow a general course parallel with the base. The standard deviations for each of these given ages are therefore constant to all intents and purposes. Since one effect of selection is to reduce the standard deviation of the selected race in

TABLE 23

Correlation surface for amount of eight-months milk production at three years old and amount of eight-months milk production at ten years and older for pure-bred Jersey cows.

10-and-above years old	3 years old: 2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	Total
3000-3500	1	1									2
3500-4000											0
4000-4500			1			1	1	1	1		5
4500-5000		1	1			1					3
5000-5500				1		2	1	1			5
5500-6000						1		1			2
6000-6500											0
6500-7000									1	1	2
Total	1	2	2	1	0	5	2	3	2	1	19

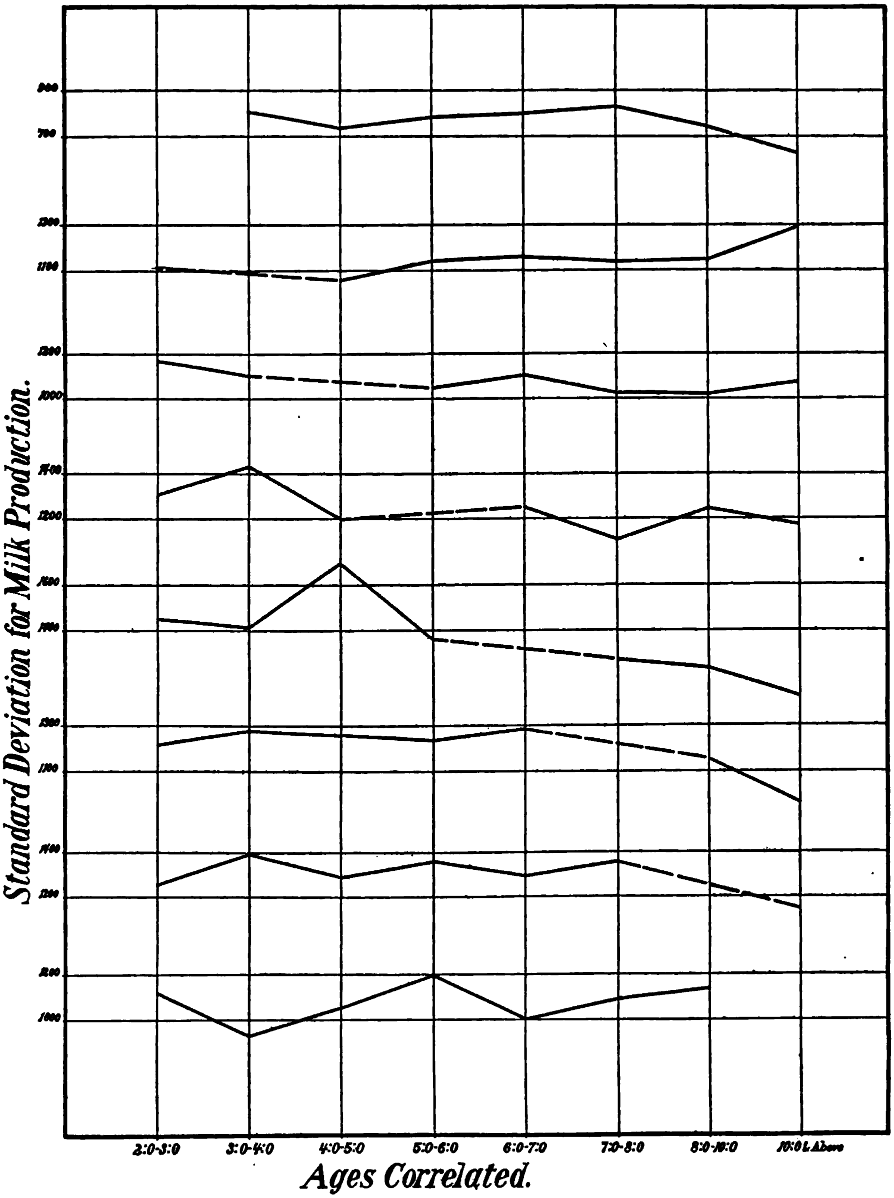

FIGURE 7.—Curves showing the standard deviations for eight-months milk production of a given age in the cow's life at the successive ages with which the given age is correlated. The ordinates are the standard deviations for the pounds of milk produced. The abscissae are the ages of the groups with which the given age is correlated and for which the given age had the standard deviation plotted. Proceeding from top to bottom the curves show the standard deviation for the 2, 3, 4, 5, 6, 7, 8-and-9 and 10-year-and-older age groups. The lines are quite regular and in general are parallel with the base as would be expected if no selection were practiced.

comparison with that of the unselected race, it follows that no selection is evidenced in these age groups. The case for the six-year group is not so clear. Here the general trend of the curve seems downward. This downward trend is only slight as compared with its probable error. The reason for it is as obscure as in the similar trend of the mean-8-months-production curve.

TABLE 24

Correlation surface for amount of eight-months milk production at four years old and amount of eight-months milk production at five years old for pure-bred Jersey cows.

4 years old

5 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	Total
3000-3500	1	1	1	2	1	1	1						8
3500-4000		1	6	5	1	3		1					17
4000-4500		3	5	2	3	2	1				1		17
4500-5000			4	8	6	6	2	1	2				29
5000-5500			1	3	6	9	4		1				24
5500-6000			1	2		4	2	2					11
6000-6500				2	2	2	3	3		2			14
6500-7000					2	2	2		3	1			10
7000-7500				1	1	1		1	2		1		7
7500-8000						1	2		1				4
8000-8500							1					1	2
Total	1	5	18	25	22	31	18	8	9	3	2	1	143

The general conclusion indicated by these data is that no selection of the high-producing cows to retain only the high milker in the older ages based on their earlier-recorded production has been practiced.

The coefficients of variation of milk production as influenced by the time the cow is retained in the herd

The relative measure of the effect of any change which may have occurred is given by the coefficients of variation for the milk production of any given age. The constants for this comparison are given in table 25. The arrangement of them in this table is identical with that for tables 11 and 20. The age for which the coefficients of variation are determined is given above the columns. The ages at which the cows have records at other ages are given on the left. The data for these are calculated from correlation tables 9 to 40.

TABLE 25

Coefficients of variation of milk production of a given year when correlated with other given years.

Age	2 years	3 years	4 years	5 years
2 to 3		23.13 ± .85	23.50 ± .87	23.57 ± 1.02
3 to 4	19.49 ± .69		21.94 ± .89	26.71 ± 1.15
4 to 5	18.00 ± .67	22.74 ± .93		23.02 ± .96
5 to 6	18.73 ± .79	23.98 ± 1.01	20.80 ± .87	
6 to 7	19.02 ± .85	24.01 ± 1.13	21.46 ± .95	23.16 ± 1.04
7 to 8	20.01 ± 1.04	23.38 ± 1.17	19.97 ± .93	20.86 ± .92
8 to 10	18.33 ± .85	24.42 ± 1.17	20.14 ± .89	23.39 ± .96
10 and older	14.93 ± 1.16	24.95 ± 2.90	21.51 ± 1.57	21.48 ± 1.41

TABLE 25 (continued)

Coefficients of variation of milk production of a given year when correlated with other given years.

Age	6 years	7 years	8 and 9 years	10 years and older
2 to 3	25.26 ± 1.14	22.38 ± 1.15	24.65 ± 1.21	22.76 ± 1.82
3 to 4	24.82 ± 1.18	23.48 ± 1.17	27.28 ± 1.34	19.00 ± 2.15
4 to 5	30.28 ± 1.42	23.72 ± 1.09	24.95 ± 1.14	22.10 ± 1.57
5 to 6	24.20 ± 1.09	22.38 ± .97	26.02 ± 1.10	24.62 ± 1.71
6 to 7		24.15 ± 1.09	24.02 ± 1.04	20.43 ± 1.24
7 to 8	23.61 ± 1.09		26.68 ± 1.12	22.64 ± 1.34
8 to 10	23.02 ± .95	22.22 ± .89		23.28 ± 1.04
10 and older	20.59 ± 1.31	20.42 ± 1.15	22.18 ± .99	

To facilitate comparison of the constants for one age with those of another age the values of the coefficients are represented as curves in figure 8. The arrangement of these curves is entirely similar to that of figures 6 and 7.

The values of the coefficients of variation are represented as ordinates and the ages at which the cows had another lactation as abscissae. The curves progressing from top to bottom of the figure are for ages 2, 3, 4, 5, 6, 7, 8 and 9, and 10 and above. The same reasoning, that if no selection of animals based on their records of previous years has taken place the curves generated by these coefficients of variation should be straight lines parallel with the base, still holds.

The curves in figure 8 are individually somewhat more irregular than the curves for the mean milk production of a given age (figure 6) and the standard deviations of a given age (figure 7). No definite trend of the coefficients either toward a higher value or toward a lower value is seen in the coefficients except those for the six-year period. This six-

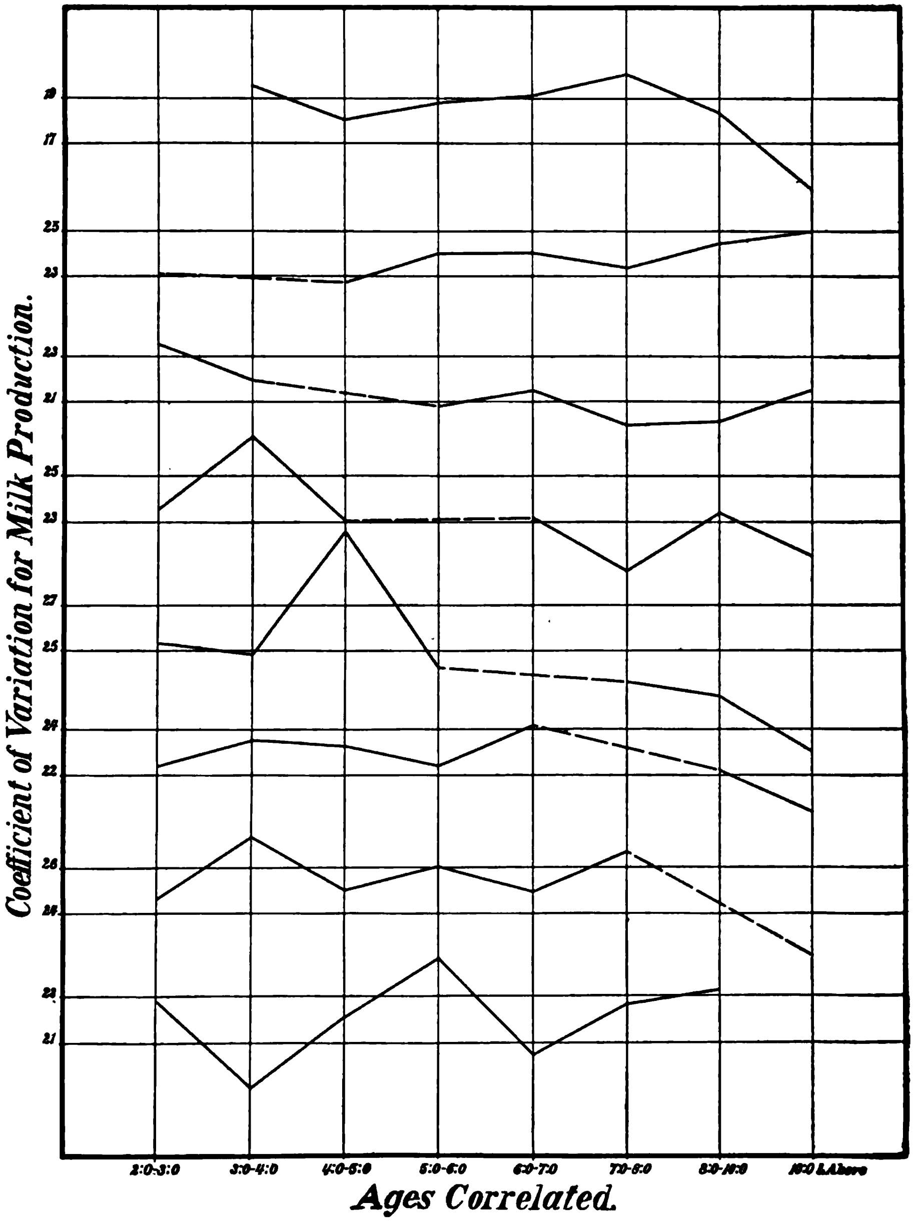

FIGURE 8.—Curves showing the coefficients of variation for the eight-months production of a given age at each successive age with which the milk yield of the given age is correlated. The ordinates are the coefficients of variation and the abscissae are the ages of the groups with which the given age is correlated. The significance of the curves proceeding from top to bottom is the same as in figures 6 and 7. The lines generated by the curves should, of course, as in the preceding case be parallel with the base and linear if no effect of selection of cattle based on their previous records has taken place.

TABLE 26

Correlation surface for amount of eight-months milk production at four years old and amount of eight-months milk production at six years old for pure-bred Jersey cows.

6 years old \ 4 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	Total
2500-3000	1							1						2
3000-3500		1		2		1								4
3500-4000		1	4	3		1								9
4000-4500			3	4	1	2								10
4500-5000			2	3	6	2	1		1					15
5000-5500		2	1	3	2	4	2	1		1				16
5500-6000		1		2	4	4	2	1	1	1	1			17
6000-6500				2	4	6	6	3	1	1				23
6500-7000				1	1	1	2		1	1	1			8
7000-7500				1	2	1	1		1					6
7500-8000						1			3					4
8000-8500							1	1	1					3
8500-9000														0
9000-9500						1		1						2
9500-10000														0
10000-10500													1	1
Total	1	5	10	21	20	24	15	8	9	4	2	0	1	120

TABLE 27

Correlation surface for amount of eight-months milk production at four years old and amount of eight-months milk production at seven years old for pure-bred Jersey cows.

7 years old \ 4 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	Total
2000-2500		1										1
2500-3000	1											1
3000-3500			1	2	1							4
3500-4000		1	2	4	2	1						10
4000-4500				5	2	2	1					10
4500-5000			5	4	3	3						15
5000-6000			2	2	2	6	3	3	1			19
5500-6000				1	7	4	2	2		1		17
6000-6500				2	3	1	3	3	1	1		14
6500-7000					2	3			3	2		10
7000-7500							1	1	1	2	1	6
7500-8000							1		1			2
8000-8500						2	1					3
8500-9000							1					1
Total	1	2	10	20	22	22	13	9	7	6	1	113

year curve is quite irregular. The general tendency is slightly downward indicating if it proves significant some selection on the basis of the

Table 28

Correlation surface for amount of eight-months milk production at four years old and amount of eight-months milk production at eight and nine years old for pure-bred Jersey cows.

8 and 9 years old

4 years old	2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	Total
2500-3000		1	1															2
3000-3500				1														1
3500-4000			2	3	1	2	3	1										12
4000-4500	2		2	2	5	3	6		4	2								26
4500-5000			1	1	5	3	8	1	1	3	2							25
5000-5500			4	2		2	4	1	2	1	1							17
5500-6000					2	4	4	2		4		1	1					18
6000-6500						2	3	3		1	1							10
6500-7000					2		1	2									1	6
7000-7500							2			1	1							4
7500-8000								1			1							2
Total	2	1	10	9	15	16	31	11	7	12	6	1	1	0	0	0	1	123

Table 29

Correlation surface for amount of eight-months milk production at four years old and amount of eight-months milk production at ten years and older for pure-bred Jersey cows.

4 years old

10-and-above years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	Total
2000-2500				1							1
2500-3000						1					1
3000-3500	1	1	1	2							5
3500-4000				1		1	1				3
4000-4500			1	3	3	1	1	1		1	11
4500-5000			1	1		2				2	6
5000-5500		1		1	3	3	2			1	11
5500-6000					2		1		1	1	5
6000-6500				1	1	2					4
6500-7000				1							1
7000-7500				1							1
Total	1	2	3	12	9	10	5	1	1	5	49

six-year milk production. Neglecting the rise at four years as obviously extraneous to the general trend of the curve, the difference between the six-year coefficients of variation for cows which were milked at 2 years and the six-year coefficients of variation for cows which were milked at 10 years is 4.67 ± 1.74, or the divergence is 2.7 times its probable error. The difference is therefore doubtfully significant, in fact probably not significant.

The conclusion which may be derived from these data is that no effect of selection of cows on the basis of their milk records is manifested by the coefficients of variation for any given age taken in conjunction with the length of time the cows remain as milkers in the herd.

TABLE 30

Correlation surface for amount of eight-months milk production at five years old and amount of eight-months milk production at six years old for pure-bred Jersey cows.

6 years old	5 years old: 3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	Total
2500-3000	1	1											2
3000-3500	2												2
3500-4000		4	3	2									9
4000-4500	2	4	2	5	1								14
4500-5000	1		1	4	5	3							14
5000-5500	1	2	1	4	5	4		1	1				19
5500-6000		2	1	1	7	1	1	3	1				17
6000-6500			1	1	3	2	5	3	1				16
6500-7000					1	1	4	2					8
7000-7500				1	1	1		2	2	1	1	1	10
7500-8000								2	3		1		6
8000-8500					1				1				2
8500-9000													0
9000-9500						1			1				2
9500-10000									1				1
Total	7	13	9	18	24	13	10	13	11	1	2	1	122

Among the important general conclusions coming out of this study of the mean 8-months milk production, the standard deviation and coefficients of variation of this milk production within the different age groups determined separately for the other ages at which they had lactations in the herd, perhaps the most important is that the data for which the curve of mean milk production with age (figure 4) was obtained are free

TABLE 31

Correlation surface for amount of eight-months milk production at five years old and amount of eight-months milk production at seven years old for pure-bred Jersey cows.

7 years old \ 5 years old	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	Total
2000-2500		1										1
2500-3000	1											1
3000-3500	1	1										2
3500-4000	1	1	1	2		3	1					9
4000-4500	2	2	1	4	4							13
4500-5000		2	6	4	5	3		1				21
5000-5500	2	2	2	4	5	4	1	2	1			23
5500-6000			1	2	4	2	2					11
6000-6500				3	5	3	4	5	1			21
6500-7000			1	2	2	1	2	2			1	11
7000-7500							3	1	3		1	8
7500-8000		1			2	1						4
8000-8500						1	1		1			3
8500-9000										1		1
Total	7	10	12	21	27	18	14	11	6	1	2	129

from any error due to selection of cows that were to remain in the herd on the basis of the records they made in their first, second or later lactations. From this it follows that the rise and fall of the curve for mean milk production with advancing age is due strictly to the physiological changes brought about in the mammary functions of the cow by age. The general equation to this physiological change expresses the law by which it is governed in the same way that MINOT (1891, 1907), LEWENZ and PEARSON (1904), DONALDSON (1908, 1909, 1910, 1911), HATAI (1911), and PEARL with the assistance of PEPPER (1907), have expressed the similar law for the manner in which the metabolic functions producing growth change with increasing age.

Added significance is given by this analysis to the conclusions previously indicated concerning the variation of milk within a given age, the correlation of age and milk production and the change of the variability of milk production with age.

TABLE 32

Correlation surface for amount of eight-months milk production at five years old and amount of eight-months milk production at eight and nine years old for pure-bred Jersey cows.

5 years old

8 and 9 years old	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	Total
2000-2500		1						1					2
2500-3000	2	1				1							4
3000-3500	3	1	1		1								6
3500-4000	3	5	1	1	3		2	1					16
4000-4500	2	2	4	4	4	3	1						20
4500-5000	1		2	2	7	3	1	1					17
5000-5500			4	4	4	4	2	1	1				20
5500-6000		2	3	2	4	3	2		2				18
6000-6500		1	1	1	6	4	1	2	2	1	1		20
6500-7000			1	1	1	1	3	4			2		13
7000-7500							1		2	1			4
7500-8000				1									1
8000-8500											1	1	2
8500-9000													0
9000-9500													0
9500-10000							1						1
10000-10500						1							1
Total	11	13	17	16	30	20	14	10	7	2	4	1	145

TABLE 33

Correlation surface for amount of eight-months milk production at five years old and amount of eight-months milk production at ten years and older for pure-bred Jersey cows.

5 years old

10 years and older	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	Total
2000-2500		1									1
2500-3000		1		1							2
3000-3500	2	1		1	1						5
3500-4000	1				1			3			5
4000-4500		1		2	1	1	1		1		7
4500-5000		1	2	1	1	2	2	1			10
5000-5500			3		1	4	2	1			11
5500-6000			1			1	2	1			5
6000-6500					1		1				2
6500-7000						1			3	1	5
7000-7500							1				1
7500-8000							1				1
Total	3	5	6	5	6	9	10	6	4	1	55

The correlation of eight-months milk production at a given age with the eight-months milk production at any other given age

For a firm foundation of our practical agriculture, particularly dairying, knowledge of the inter-relationships of the workings of the mammary functions at one age in comparison with its precision of action at another age can scarcely be too exact. The existing practice is, as already pointed out, largely empirical in its nature, often leading to questionable results. The reflection of the questionable nature of these practices is seen in the not uncommon practice of dairymen neglecting the records of the first lactation as a measure of the cow's possibilities for future production.

TABLE 34

Correlation surface for amount of eight-months milk production at six years old and amount of eight-months milk production at seven years old for pure-bred Jersey cows.

7 years old \ 6 years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	Total
2000-2500			1	1											2
2500-3000	1														1
3000-3500		1	2	2											5
3500-4000		2	4	2	1	1		2							12
4000-4500			1	4	2	3		1	1						12
4500-5000			3	2	6	5	2								18
5000-5500	1		1	4	1	5	3	2	3			2			22
5500-6000			1	1	1	2	3	4	1	1					14
6000-6500				1		3	2	7		1	1				15
6500-7000					1	1	3	3		2	1				11
7000-7500					1		1	1	1	1	1				6
7500-8000							1					1			2
8000-8500								1			1			1	3
8500-9000										1					1
Total	2	3	13	17	13	20	15	21	6	6	4	3	0	1	124

The correlations between the milk production of various lactations with one another have material importance as well as theoretical interest for these problems. If the correlation is high it is evident from the practical side that the culling of the herd through the use of dependable criteria will result in the increase of profits to its owners. From the biological side, a high correlation means that the animals composing the herd are innately differentiated (presumably due to their inherited complex) in their mammary capacities.

TABLE 35

Correlation surface for amount of eight-months milk production at six years old and amount of eight-months milk production at eight and nine years old for pure-bred Jersey cows.

6 years old	8 and 9 years old: 2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-1000	Total
2500-3000		1	1				1	1									4
3000-3500		1	2	2													5
3500-4000			1	2	4	3	1										11
4000-4500	1		2	2	1		2	1	3	1	1						14
4500-5000		2	4	1	3	6	6	1	3	2							28
5000-5500		1	1	2	1	4	4		3	2	1	1					20
5500-6000					3	2	5	3	5	1	1						20
6000-6500	1				3	2	6	4		3	1						20
6500-7000						1	3	1		3						1	9
7000-7500						1	2	2	4	1			1				11
7500-8000										1	1						2
8000-8500							1	1		1	1						4
Total	2	5	11	9	15	19	31	14	18	15	6	1	1	0	0	1	148

The determination of the correlations for these data is of especial interest, for, as previously shown in the earlier part of this paper, the data are of exceptional value in that they are on a pure-bred Jersey herd kept intact for many years; the lactation records for several lactations are recorded on a number of the animals; and the herd has been subject to no detectable culling based on the production of a given lactation. From these data there have been extracted lactation records to the number of 3178 pairs having a full eight-months lactation free from any disease or sickness or other trouble known to influence the records. These records have been formed into twenty-eight correlation tables the most of which are of considerable size.

After careful consideration it was decided that since these tables have much of scientific and practical value to the practice of dairy husbandry they had better be printed *in toto*. These tables are reproduced in the text tables numbers 9 to 40.

The means, standard deviations and coefficients of variation for these correlation tables have been presented in tables 11, 20 and 25. Table 32 gives the correlations and their probable errors for all ages at which the lactation records were divided. The vertical columns give the correlations of the milk production at the age heading the column with the

TABLE 36

Correlation surface for amount of eight-months milk production at six years old and amount of eight-months milk production at ten years and older for pure-bred Jersey cows.

6 years old

10-and-above years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	Total
2000-2500			1								1
2500-3500						1					1
3000-3500	1	1		1		1					4
3500-4000			2			1		2			5
4000-4500			1		2		3	1		1	8
4500-5000			2	1	4	2	2	3		1	15
5000-5500		1	1		2	1	3	5	1		14
5500-6000					4		1	2			7
6000-6500				1				3	1		5
6500-7000										3	3
7000-7500								1			1
Total	1	2	7	3	12	6	9	17	2	5	64

TABLE 37

Correlation surface for amount of eight-months milk production at seven years old and amount of eight-months milk production at eight and nine years old for pure-bred Jersey cows.

8 and 9 years old

7 years old	2000-2500	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	7500-8000	8000-8500	8500-9000	9000-9500	9500-10000	10000-10500	Total
2500-3000		1	1															2
3000-3500	1	1	1	2	2		1											8
3500-4000			3	3	5	3	3											17
4000-4500	1	1		3	1	2	4	1		1								14
4500-5000		1	3	5	2	6	5	2	2	1								27
5000-5500		1		2	3	2	5	3	3	2	2							23
5500-6000				1	1	2	6	1	3	1	3							18
6000-6500	1				4	4	2	1	3	2								17
6500-7000					2	1	3	4	2	2	1							15
7000-7500							1	1	1	1	1	1	1			1		8
7500-8000										1							1	2
Total	3	5	8	16	20	20	30	13	14	11	7	1	1	0	0	1	1	151

milk production at the ages indicated at the left-hand margin of the table.

As will be noted the correlations necessary to give the complete set of correlations for any given age are repeated, e.g., the correlation of milk production at 2 years with that at 3 years is +0.5764±.0332 and appears in the 2-year column. The correlation of the 3-years milk production with that of 2 years will, of course, be the same (0.5764±.0332) and is repeated in the three-year column. In this manner a complete picture of the relationship of the lactations of the 2-year groups with the other lactations of the other groups is given in the column.

Table 38

Coefficients of correlation of milk production of a given age with the milk production of another given age.

Age	2-3 years	3-4 years	4-5 years	5-6 years
2 to 3		+0.5764 ± .0332	+0.5426 ± .0361	+0.5373 ± .0406
3 to 4	+0.5764 ± .0332		+0.6206 ± .0335	+0.5479 ± .0395
4 to 5	+0.5426 ± .0361	+0.6206 ± .0335		+0.5541 ± .0391
5 to 6	+0.5373 ± .0406	+0.5479 ± .0395	+0.5541 ± .0391	
6 to 7	+0.5500 ± .0426	+0.5305 ± .0452	+0.5624 ± .0421	+0.7306 ± .0284
7 to 8	+0.5815 ± .0468	+0.5394 ± .0483	+0.6328 ± .0380	+0.5667 ± .0403
8 to 10	+0.4938 ± .0488	+0.5376 ± .0465	+0.4154 ± .0503	+0.5405 ± .0397
10 and older	+0.5603 ± .0732	+0.6336 ± .0925	+0.2144 ± .0919	+0.5371 ± .0697

Table 38 (continued)

Coefficients of correlation of milk production of a given age with the milk production of another given age.

Age	6-7 years	7-8 years	8-10 years	10 years and older
2 to 3	+0.5500 ± .0426	+0.5815 ± .0468	+0.4938 ± .0488	+0.5603 ± .0732
3 to 4	+0.5305 ± .0452	+0.5394 ± .0483	+0.5376 ± .0465	+0.6336 ± .0925
4 to 5	+0.5624 ± .0421	+0.6328 ± .0380	+0.4154 ± .0503	+0.2144 ± .0919
5 to 6	+0.7306 ± .0284	+0.5667 ± .0403	+0.5405 ± .0397	+0.5371 ± .0697
6 to 7		+0.6515 ± .0349	+0.4800 ± .0427	+0.4578 ± .0666
7 to 8	+0.6515 ± .0349		+0.5750 ± .0367	+0.3036 ± .0712
8 to 10	+0.4800 ± .0427	+0.5750 ± .0367		+0.5113 ± .0448
10 and older	+0.4578 ± .0666	+0.3036 ± .0712	+0.5113 ± .0448	

The order of magnitude of these correlations is from +0.7306 for the correlation of 8-months milk productions during the ages 5 to 6 and 6 to 7 years, and +0.2144 for the correlation of the productions during the ages 4 to 5 and 10 years and older. Such correlations indicate that the milk production of one lactation may be predicted with relatively little inaccuracy from the milk production of another lactation. The graphs showing these correlations for each year are seen in figure 9.

The arrangement of this figure is similar to that of figure 6. The magnitudes of the correlations are represented as ordinates. The ages of the cows whose productions are correlated with those of the given age are represented as abscissae. The curves progressing from top to bottom of the figure represent the correlations of the 8-months milk productions at the ages two, three, four, five, six, seven, eight and nine, and over ten years with the eight-months productions occurring at the other ages shown on the lower margin of the figure.

The graph for the correlation coefficients of the milk production at two years with the milk production of the subsequent lactations of the same cows shows little deviation from a straight line. The values of these correlations range from +0.4938±.0488 to +0.5815±.0468. The values for the three-year correlations with the other years range somewhat higher, +0.5305±.0452 to +0.6336±.0925. These correlations would be quite accurately described by a linear function. The values of the correlation of the four-year-olds' production with those of other years range from +0.2144±.0919 to +0.6328±.0380. On the whole these values are slightly lower than are those for other years. The values for the five-year-olds range from +0.5371±.0697 to +0.7306±.0284. These values are the highest in their range of any of the ages. The correlations for the six-year productions with those of other ages range from 0.4578±.0666 to +0.7306±.0284. The correlations for the seven-year productions range from 0.3036±.0712 to +0.6515±.0349. The correlations for the 8-and-9-year period range from +0.4154±.0503 to +0.5750±.0367 and the correlations for the productions at 10 years and older with those of the younger years range from +0.2144±.0919 to +0.6336±.0925. From this brief resumé of the tabled results it is seen that all of the results have the plus sign and have a rather large value for data of this kind. The values of the coefficients are in general higher for the younger ages than they are for the older ages. This is a fortunate circumstance from a practical standpoint for the breeder is more desirous of selecting the animals to remain in the herd from their two-year records than he is to select his animals at ten years old. The values of the correlations are fortunately such that a gain in accuracy results by predicting from the two-year-olds as over predictions at later ages.

From the practical side of culling the poor milkers out of the herd these results are highly satisfactory. The data are equally interesting when considered in their biological aspects. The correlations show that the cows composing the herd are innately differentiated in their milk-

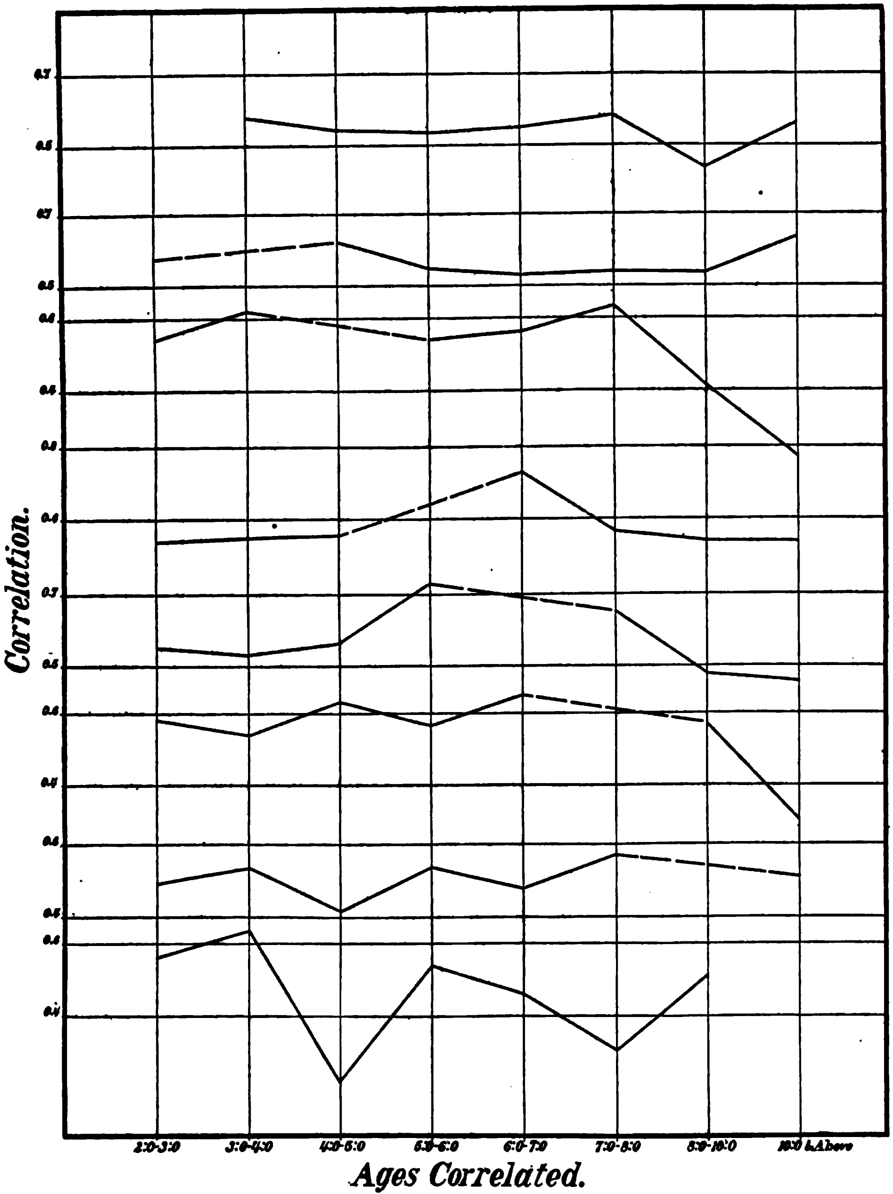

FIGURE 9.—Curves showing the coefficients of correlation of milk production of a given age and the milk production occurring at any other given age. The ordinates are the values of the correlation coefficients. The abscissae are the ages at which the lactation record compared with the record at the given age, was made. Thus the top curve of the figure shows the correlations of records made at two years old with the records made at subsequent ages. From this curve the correlation between two-years milk production and three-years milk production is found on the three-year line of the two-year curve (top curve of figure) to be 0.5764. The correlation of the two-year and four-year record is found on the four-year line where the two-year line crosses it (top curve of figure) to be 0.5426, etc. The dotted lines serve to connect the correlations immediately preceding and following the age for which the line is drawn.

Table 39

Correlation surface for amount of eight-months milk production at seven years old and amount of eight-months milk production at ten years and older for pure-bred Jersey cows.

7 years old

10-and-above years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	Total
2000-2500					1						1
2500-3000				1			1				2
3000-3500	1		1		1	3		1			7
3500-4000		1		1	1	2					5
4000-4500			1	1	2	2	4	1			11
4500-5000		1	2	4	3	1	3	1	1	1	17
5000-5500		1	4		2		3	2	1	1	14
5500-6000					1		2	1	1	2	7
6000-6500						1	4				5
6500-7000						1	1				2
7000-7500				1				1			2
7500-8000									1		1
Total	1	3	8	8	11	10	18	7	4	4	74

Table 40

Correlation surface for amount of eight-months milk production at eight and nine years old and amount of eight-months milk production at ten years old and older for pure-bred Jersey cows.

8 and 9 years old

10-and-above years old	2500-3000	3000-3500	3500-4000	4000-4500	4500-5000	5000-5500	5500-6000	6000-6500	6500-7000	7000-7500	Total
2000-2500		1		1							2
2500-3000			1		1	1					3
3000-3500	2	2	3	2		1		1			11
3500-4000			2	2	2				2		8
4000-4500		1	3	2	3	5		2	1	1	18
4500-5000	1	1	5	2	4	10	2		2		27
5000-5500			2	3	2	6		5	4	1	23
5500-6000				1		3	2	2	5		13
6000-6500						1		1	1	2	5
6500-7000						5	1	2		1	9
7000-7500						1		1	1		3
7500-8000							1		1		2
Total	3	5	16	13	12	33	6	14	17	5	124

producing abilities. The plane of producton once established the cow tends to maintain this relative plane from lactation to lactation. The values of the correlations indicate clearly that there is a fairly accurately working mechanism behind this function.

From this point of view it is of a good deal of interest to compare the only other statistics available for cattle, with those derived here. GAVIN (1913) using his measure of the lactation as the "revised maximum" found the correlation between the milk productions of the various lactations and that of their maximum "revised maximum" GAVIN (1912). Table 41 is a copy of GAVIN's table showing these correlations.

TABLE 41

Lactation	r with maximum "revised maximum"	Probable error
First	+ .394	± 0.031
Second	+ .452	± 0.030
Third	+ .506	± 0.028
Fourth	+ .605	± 0.024
Fifth	+ .762	± 0.016

This table shows in general a lower value for the correlations measuring the consistency of milking performance from lactation to lactation than is shown by our data. This may in part be due to the fact that GAVIN's material was somewhat heterogeneous, including data from British Holsteins and Shorthorns. In all events, the correlations confirm our general conclusions that milk production of one lactation is quite closely correlated with that of the other lactations. This reasoning transferred to the individuals of the race of dairy cattle, appears to prove beyond any shade of doubt that the individuals of the race are innately differentiated as regards the capacity for milk production.

Of the quantitative data on other species of practical interest perhaps the most complete is that of HARRIS and BLAKESLEE (1918) on the White Leghorn. In this work they determined the correlations between the monthly egg production and the other-eleven-months production of the same bird. The correlations for these monthly ovulations with the other-eleven-months ovulation take values ranging from +0.240±.033 to +0.573±.023. The range is there quite similar to those obtained in this although lower in value. The knowledge of these sets of constants gives criteria for the fairly accurate prediction of the records that may be expected at a subsequent date in the life of these two species of extreme economic importance.

THE REGRESSION OF THE MILK PRODUCTIONS OF THE DIFFERENT LACTATIONS ON EACH OTHER

The constants necessary to determine the approach of the regression to linearity are given in table 42. The values of the correlation coefficients have been repeated for greater ease in drawing conclusions from the values of the correlation ratios.

In this table the values of the correlation ratios range from 0.4382 to 0.8189. The conclusions drawn from a study of the correlation coefficients is thus confirmed by these correlation ratios.

As is generally well recognized, the coefficients of correlation depend for their accuracy on the regression lines being closely described by a linear function. The values of the correlation ratio on the other hand do not depend on this fitting of linear functions to the rough regression lines but do give a measure of the association from the regression line itself. If this rough regression is linear the two constants should agree within the limits of their probable errors. In table 42 columns $\eta—r$ and $\eta^2—r^2$ give constants to measure this agreement. The last column gives the multiple number of times $\eta^2—r^2$ is to the probable error. This column gives us in a form easily appreciated the measure of what significance can be attached to these constants. The values range from 0.33 to 4.46, from an insignificant deviation to one probably significant. Of the 28 values only six are less than 2 times their probable error. The average of the 28 constants shows that the mean deviation is 2.42 times the probable error. On the face of this it would seem that there is a slight deviation of the regression lines from the linear. This deviation may be due to the duration of life covered by the age units within which the lactation records were correlated. These units were in general of one year in length. During this time, as was shown earlier in this paper, there is a non-linear rise in milk production. One year appears to be of sufficient length of time to have produced this effect in the milk yield. To this effect the appearance of a regression differing slightly from the linear may be attributed. It would appear that should a shorter unit of time be chosen, the value of the correlation coefficients would agree more closely with those of the correlation ratios.

This reasoning, while apparently probable, does not prove true, as a later section of the paper dealing with regressions over five lactations shows. This slight lack of linearity must therefore be considered due either to chance or to some obscure causes. In any case it will not influence the results materially where the correlations are used as constants for calculation.

lk from lactation to lactation.

	$\eta - r$	$\eta^2 - r^2$	$\frac{\eta^2 - r^2}{E_{\eta^2 - r^2}}$
2 years	512 ± .0448	0.0615 ± .0239	2.58
“	496 ± .0490	.0563 ± .0236	2.39
“	976 ± .0530	.1143 ± .0363	3.15
“	706 ± .0568	.0826 ± .0336	2.46
“	792 ± .0614	.0984 ± .0421	2.34
“	095 ± .0638	.1201 ± .0420	2.86
“	478 ± .0905	.1875 ± .0832	2.25
3 years t	86 ± .0407	.1912 ± .0429	4.46
“	169 ± .0505	.1418 ± .0393	3.60
“	823 ± .0599	.0942 ± .0368	2.56
“	987 ± .0630	.1162 ± .0437	2.66
“	707 ± .0622	.0810 ± .0358	2.27
“	853 ± .1056	.2691 ± .1409	1.91
4 years t	120 ± .0547	.0134 ± .0411	.33
4	562 ± .0567	.0664 ± .0306	2.17
4	835 ± .0490	.1127 ± .0401	2.81
4	205 ± .0664	.1146 ± .0387	2.96
“ “	208 ± .1148	.2406 ± .0824	2.92
5 years t	246 ± .0387	.0366 ± .0229	1.59
“ “	971 ± .0522	.1195 ± .0385	3.10
“ “	212 ± .0507	.1457 ± .0395	3.69
“ “	445 ± .0809	.1761 ± .0693	2.54
6 years to	198 ± .0482	.0261 ± .0193	1.35
“ “	585 ± .0480	.0596 ± .0262	2.27
“ “	145 ± .0875	.1178 ± .0544	2.17
7 years to	077 ± .0515	.0089 ± .0103	.86
“ “	346 ± .0953	.0998 ± .0470	2.12
8 and 9 ye older	43 ± .0622	.0253 ± .0190	1.33

From these correlation coefficients in tables 41 and 42 it is possible to form the straight-line prediction of the milk yield of any age from the yield of any other age. As emphasized repeatedly in the various sections of this paper dealing with the separate subjects, the predictions may be criticised on the following grounds: that the mean milk yield rises with age in a line described by a logarithmic function; that the standard deviation of this milk yield rises in line described by a cubic parabola; and that the values of the correlation coefficients differ from the values of the correlation ratios by 2.42 times the probable error of $\eta^2 - r^2$. Such criticisms are recognized and admitted at once. It is believed that even admitting these there are a number of important points which may be elucidated by these equations.

The general equation for these regressions is given by

$$Y = \left(\text{Mean}_Y - r_{Yy} \frac{\sigma_Y}{\sigma_y} \text{Mean}_y\right) + r_{Yy} \frac{\sigma_Y}{\sigma_y} y$$

where y = 8-months milk yield in a given year and Y the expected milk yield for the year desired. The results of the substitution of the values of tables 11 to 42 in this equation are given in table 43.

As the milk production is given in pounds the second term of each of these equations gives the gain in expected milk yield for the given age, if one pound increase in actual production is made during the test. The calculation of an expected yield is, therefore, a simple matter of direct substitution.

Before discussing the relative practical and theoretical merits of these equations for the study of milk records it may be well to take up the equations individually. The coefficients of the y's (actual yields) represent the reliance that may be put on these actual yields for subsequent productions. The information desired by those who use these coefficients for actual records may be divided into two heads: (1) At what age should the cow make her record to give the best index of her potential producing abilities? (2) To what age should the records, made at different periods in the life of the cow, be corrected so that the maximum reliance may be placed on the corrected record?

The first of these questions entails a study of the coefficients of the y's for the ages at which the record was made. The data for this are given in table 44. The equations in this table are of no value save for this comparison, for if any record is to be corrected the equations in table 43 should be used.

TABLE 43

Regression equations of the milk yield for any age from that of any lactation record at another age.

Age at which milk yield was made	Age at which expected milk yield is desired			
	2 years to 3 years	3 years to 4 years	4 years to 5 years	5 years to 6 years
	Regression equation	Regression equation	Regression equation	Regression equation
2 years to 3 years y_2		$Y = 1515.5 + .7922y_2$	$Y = 1447.4 + .8591y_2$	$Y = 1795.7 + .8968y_2$
3 years to 4 years y_3	$Y = 2130.0 + .4194y_3$		$Y = 2013.7 + .6500y_3$	$Y = 2082.5 + .6882y_3$
4 years to 5 years y_4	$Y = 2391.2 + .3427y_4$	$Y = 1656.2 + .5935y_4$		$Y = 2007.2 + .6325y_4$
5 years to 6 years y_5	$Y = 2394.4 + .3219y_5$	$Y = 2408.3 + .4362y_5$	$Y = 2513.3 + .4855y_5$	
6 years to 7 years y_6	$Y = 2456.2 + .3018y_6$	$Y = 2346.6 + .4330y_6$	$Y = 3092.2 + .3652y_6$	$Y = 1624.4 + .6708y_6$
7 years to 8 years y_7	$Y = 1995.3 + .3955y_7$	$Y = 2252.6 + .4790y_7$	$Y = 2345.0 + .5149y_7$	$Y = 2507.8 + .5086y_7$
8 years to 10 years y_8	$Y = 2570.8 + .2927y_8$	$Y = 2437.6 + .4433y_8$	$Y = 3367.9 + .3298y_8$	$Y = 2715.2 + .4959y_8$
10 years and above y_{10}	$Y = 2633.1 + 3105y_{10}$	$Y = 868.8 + .8766y_{10}$	$Y = 3952.6 + .2190y_{10}$	$Y = 2905.2 + .5265y_{10}$

TABLE 43 (continued)

Regression equations of the milk yield for any age from that of any lactation record at another age.

Age at which milk yield was made	Age at which expected milk yield is desired			
	6 years to 7 years	7 years to 8 years	8 years to 10 years	10 years and above
	Regression equation	Regression equation	Regression equation	Regression equation
2 years to 3 years y_2	$Y = 1551.7 + 1.0023y_2$	$Y = 1910.8 + .8549y_2$	$Y = 1710.8 + .8329y_2$	$Y = 717.0 + 1.010y_2$
3 years to 4 years y_3	$Y = 2578.2 + .6500y_3$	$Y = 2502.2 + .6075y_3$	$Y = 2036.3 + .6519y_3$	$Y = 2539.6 + .4580y_3$
4 years to 5 years y_4	$Y = 1158.8 + .8662y_4$	$Y = 1435.1 + .7778y_4$	$Y = 2500.7 + .5233y_4$	$Y = 3711.3 + .2100y_4$
5 years to 6 years y_5	$Y = 1332.7 + .7958y_5$	$Y = 2165.0 + .6314y_5$	$Y = 2087.7 + .5890y_5$	$Y = 1871.7 + .5480y_5$
6 years to 7 years y_6		$Y = 1779.2 + .6585y_6$	$Y = 2493.0 + .5019y_6$	$Y = 2681.7 + .4147y_6$
7 years to 8 years y_7	$Y = 1960.8 + .6445y_7$		$Y = 1573.3 + .6720y_7$	$Y = 3205.1 + .3129y_7$
8 years to 10 years y_8	$Y = 2989.5 + .4591y_8$	$Y = 2720.5 + .4920y_8$		
10 years and above y_{10}	$Y = 2899.8 + .5053y_{10}$	$Y = 3772.8 + .2946y_{10}$	$Y = 2660.1 + .5150y_{10}$	$Y = 2271.5 + .5075y_8$

TABLE 44

Average regression equations of milk records at the same age for the expected records made throughout the life of the cow. (Summarized from table 43.)

Age at which milk record was made	Regression equation
2 years to 3 years	$Y = 1521.3 + .8927y_2$
3 " to 4 "	$Y = 2268.9 + .5893y_3$
4 " to 5 "	$Y = 2122.9 + .5637y_4$
5 " to 6 "	$Y = 2110.4 + .5440y_5$
6 " to 7 "	$Y = 2353.3 + .4780y_6$
7 " to 8 "	$Y = 2262.8 + .5039y_7$
8 " to 10 "	$Y = 2724.7 + .4315y_8$
10 years and over	$Y = 2813.2 + .4639y_{10}$

These data in table 44 show the average values of the regression equations for each age. The coefficients of the y's are the largest at the early years of the cow's life. While quite irregular the values tend to decline as the age when the lactation is made increases. Practically considered this manner of decline is very fortunate. The first lactation being the one most accurately measuring the expectation of the cow in subsequent years enables the farmer or dairymen to determine with greatest certainty from this lactation what the cow is most likely to produce. On this basis the herd may be culled of the poor producers after the first lactation in full knowledge that they would always remain relatively low milkers throughout their life time. Theoretically considered the trend of this decline is of equal interest. Most published records on all breeds of cattle have the greatest number of cattle tested when two years old. It is of considerable consequence, therefore to have the records of the two-year-olds a reasonably accurate measure of the cow's milking capacity. It is of further interest to know that these two-year-olds are the most dependable index of this producing ability.

The data in table 45 summarize the information necessary to deal with the second question. As for table 44 the equations are of no value save for the comparison in hand. If any record is to be corrected the equations of table 43 should be used.

This table shows that the highest values of the coefficients of the y's occurs for the ages 6 years to 7 years. On each side of these ages the values decline toward both the lower and higher years. Practically considered this maximum of the curve shows that the relative potentialities of the cows within the herd is most accurately determined by correcting the records of these animals to the age basis of 6 years to 7 years. This as determined previously in the paper is near the age when the maximum production of this group of Jerseys may be expected.

TABLE 45

Average regression equations of expected milk records at a given age for records at other ages throughout the life of the cow. (Summarised from table 43.)

Age for expected milk yield	Regression equation
2 years to 3 years	$Y = 2367.3 + .3406y_{x-2}$
3 " to 4 "	$Y = 1926.5 + .5791y_{x-3}$
4 " to 5 "	$Y = 2676.0 + .4891y_{x-4}$
5 " to 6 "	$Y = 2234.0 + .6313y_{x-5}$
6 " to 7 "	$Y = 2067.4 + .7033y_{x-6}$
7 " to 8 "	$Y = 2326.5 + .6167y_{x-7}$
8 " to 10 "	$Y = 2151.7 + .6123y_{x-8}$
10 years and over	$Y = 2428.3 + .4946y_{x-9}$

Before turning to other phases of the subject it may be well to discuss the practical and theoretical errors that in some measure vitiate the use of these equations given in table 43. In the practical application the equations will only give values approximately correct. The reason for this is found in an earlier section of the paper where it was shown that milk production is a highly variable character. This variation is sufficient to cause a not inconsiderable difference in the records of certain cows from lactation to lactation. The difference in these records is also expressed in the corrected expected records as calculated from these equations.

The equations are in slight error since they do not take account of the lack of linearity of regression. This difference is such as to make only a very slight error as may be judged by the fact that $\eta^2—r^2$ is on an average 2.42 times its probable error (page 164).

None of the above errors are very serious drawbacks to the actual use of their equations in practical dairying. The reason for this is simple. The main object for which these equations are of use in a practical sense (the culling the herd of the poor milkers) is little influenced by small errors as the dairyman is not going to select the animals he is going to keep on a difference of one pound. His selections will be as widely separated as possible.

In dealing with milk records from the view-point of inheritance another question should be considered, namely, what makes the regression of the different lactations toward the mean of the general population? Is it an expression of a genotype or is it variation impressed by environment on the genotype in its phenotypic expression? From the general physiological side of milk-production studies the facts all point to the explanation of this regression as principally due to the conditions not being ideal for the full expression of a cow's potentialities. A few illustrations will make this clear. The work of WING and FOORD (1904) in New York and ECKLES (1912) in Missouri, have shown that meager feeding will reduce the quantity of milk that a cow will produce in a given lactation. WOLL (1902) has reported Danish records to prove that indoor housing decreases milk yield. DOLGICH has shown that working the cows in excess decreases the milk flow. If it is admitted, as it seems it must be that these known variations influence the potential capacities of cows for milk production, it is equally likely that the whole environmental complex in which the cow lives also influences the yield. The studies of PEARL and MINER (1919) indicate these environmental influences amount on an average to as much as one-third of the observed coefficients of variation of the milk of a cow. These environmental influences undoubtedly vary from lactation to lactation and from cow to cow. Such conditions make for regression, for it is only the fortunate coincidence that favors the full expression of the cow's milking capacities. If these considerations are granted as the true state of affairs, inheritance studies of milk production certainly should disregard this regression, as the inclusion of it (as is done by the use of these regression equations) would simply befog the true mechanism if not hide it altogether. Under these circumstances the student of inheritance should use the equation of the mean curve and not the regression equation to count the records of the individual cows, if possible choosing the best record of the series of records as in all probability this represents the fullest expression of the cow's genetic constitution.

THE CORRELATION OF THE MILK PRODUCTION OF A GIVEN AGE WITH THE TOTAL PRODUCTION OF TWO LACTATIONS

This question and the further extension of it to cover the case of the cow's whole lactation history are of especial interest to the practical dairyman. He is interested primarily in the total yield he may expect from a cow rather than the yield of individual lactation.

Two sets of correlations have been determined to answer this problem; one set on the data as above given where the correlation is between the lactation record of the given year and the lactation records of the given year plus the record of some other year; the second a special set of correlations for the correlation of the summed production for the first five lactations with their component parts.

The first set of correlations may fortunately be calculated without the formation of the correlation tables from the constants already obtained for tables 11, 20 and 25. The mean and standard deviation of the sum of two components are known when $x = y + z$:

$$\overline{x} = \overline{y} + \overline{z}$$

$$\sigma_x = \sqrt{\sigma_y^2 + \sigma_z^2 + 2r_{yz}\,\sigma_y\,\sigma_z}$$

The product moments are (HARRIS 1917):

$$\Sigma\,(xy) = \Sigma\,(y^2) + \Sigma\,(yz)$$
$$\Sigma\,(xz) = \Sigma\,(z^2) + \Sigma\,(yz)$$

The calculation is then quite simple although tedious. The correlations found by this method are given in table 46.

The correlations are all very large when compared with correlations on most physiological material. They range from 0.7324±.0286 to +0.9438±.0116. Such high values make it appear that the milk production of one lactation is the determining factor for the total milk production of two lactations when the given lactation is one of the components.

This dependence is well worth testing further. For this purpose a series of data including records of five lactations was chosen. Each of the cows whose records were selected began milking in the second year. The five lactations were consecutive and approximately a year apart depending on how long the previous lactations were continued. The lactation records for the five successive lactations were correlated with the total milk production for the five lactations. The correlation coefficients and other constants for these are given in table 47. These constants are calculated from tables 48 to 53.

The high correlation between the record of one lactation and the total production (first five lactation periods) is manifest. The correlations

TABLE 47

Age when lactation commenced	Mean milk production	Standard deviation for the milk production	Coefficients of variation for the milk production
2 years to 3 years	4159.1 ± 57.8	803.2 ± 40.8	19.31 ± 1.00
3 years to 4 years	4840.9 ± 86.1	1197.9 ± 60.9	24.74 ± 1.35
4 years to 5 years	5380.7 ± 78.8	1096.2 ± 55.7	20.37 ± 1.06
5 years to 6 years	5568.2 ± 87.1	1211.2 ± 61.6	21.75 ± 1.17
6 years to 7 years	5681.8 ± 91.4	1270.7 ± 64.6	22.36 ± 1.17
Total of five lactations	25613.6 ± 335.9	4672.0 ± 237.5	18.24 ± .94

Correlati tation record of the given year plus the record

Character	Correlation
2 and (2 + + 3)	+0.9190 ± .0077
2 and (2 + + 4)	.9300 ± .0069
2 and (2 + + 5)	.9341 ± .0073
2 and (2 + + 6)	.9433 ± .0068
2 and (2 + + 7)	.9296 ± .0096
2 and (2 + d (2 + 8 and 9)	.9289 ± .0088
2 and (2 + and (2 + 10 and over)	.9438 ± .0116
3 and (3 + + 4)	.9048 ± .0099
3 and (3 + + 5)	.9071 ± .0100
3 and (3 + + 6)	.9005 ± .0118
3 and (3 + + 7)	.8924 ± .0139
3 and (3 + d (3 + 7)	.9009 ± .0123
3 and (3 + and (3 + 10 and over)	.8686 ± .0379
4 and (4 + + 5)	.8976 ± .0109
4 and (4 + + 6)	.7324 ± .0286
4 and (4 + + 7)	.9233 ± .0094
4 and (4 + d (4 + 8 and 9)	.8788 ± .0138
4 and (4 + and (4 + 10 and over)	.7739 ± .0386
5 and (5 + + 6)	.9363 ± .0076
5 and (5 + + 7)	.8979 ± .0115
5 and (5 + d (5 + 8 and 9)	.8886 ± .0117
5 and (5 + and (5 + 10 and over)	.8793 ± .0207
6 and (6 + + 7)	.9097 ± .0104
6 and (6 + (6 + 8 and 9)	.8669 ± .0138
6 and (6 + and (6 + 10 and over)	.8377 ± .0251
7 and (7 + d (7 + 8 and 9)	.9053 ± .0099
7 and (7 + and (7 + 10 and over)	.8139 ± .0265
8 and 9 and and (8 and 9 + 10 and over)	.8682 ± .0149

* Under ation record was made. The *and* signifies this lactation rec ds made at the ages indicated in the parenthesis. The *age* at in parenthesis. The *plus* sign signifies that this record was nd term of the parenthesis.

TABLE 47 (continued)

Age when lactation commenced	Correlation of individual lactation records and five-lactations total production	Correlation ratios of individual lactation records and five-lactations total production	Regression equations. Eight-months lactation and five-lactation total production
2 years to 3 years	+0.7416 ± .0323	+0.7517 ± .0312	$Y_T = 7671.9 + 4.3139y_2$
3 years to 4 years	.8418 ± .0209	.8856 ± .0155	$Y_T = 9719.5 + 3.2833y_3$
4 years to 5 years	.8613 ± .0186	.8796 ± .0162	$Y_T = 5861.4 + 3.6710y_4$
5 years to 6 years	.8250 ± .0230	.8561 ± .0192	$Y_T = 7893.5 + 3.1824y_5$
6 years to 7 years	.8205 ± .0234	.8468 ± .0203	$Y_T = 8471.9 + 3.0170y_6$
Total of five lactations			

are so high as to lead to the conclusion that the record of one lactation in a cow's life serves to determine the records for the total production in the cow's life.

The bearing of this conclusion on the mechanism behind the formation of milk is clearly of much significance. The mammary glands may be considered to function because of the interaction of the two variables environment and heredity. Environment may be considered to influence this functioning in two stages; the period during the growth of the cow up to the time she calves and the time after calving when milk is actually secreted. Since this study deals with the records of the same individual cow for successive lactations the inherited complex for her potential milk-producing capacity may be said to be given and constant throughout this work.

The highest possible milk production, or what might be considered the full expression of the hereditary complex, for a given cow may be considered as brought about by ideal environmental conditions for that cow. Taking this definition of the ideal environmental conditions it follows that environmental changes from this condition however slight they may be, are detrimental to the full expression of the mammary gland's possibilities. In a herd like the one with which these data deal no extremely detrimental conditions for any individual cow will exist. The reason for this is clear when it is remembered that the herd has always been kept in the same location under the direction of the same manager thoroughly versed in the feeding and handling of cattle.

Some measure of the influence of environment on the correlation coefficients in the third division into which the mammary functions were divided may be obtained by comparing such coefficients for one lactation period and those covering several lactations. Thus if we correlate

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TABLE 48

Correlation surface for amount of eight-months milk production for the first five lactations in the cow's life and the amount of eight-months milk production at two years old for pure-bred Jersey cows.

Total production for first five lactations (eight-months periods)

2 years old	15000-16000	16000-17000	17000-18000	18000-19000	19000-20000	20000-21000	21000-22000	22000-23000	23000-24000	24000-25000	25000-26000	26000-27000	27000-28000	28000-29000	29000-30000	30000-31000	31000-32000	32000-33000	33000-34000	34000-35000	35000-36000	36000-37000	Total
2000-2500	1																						1
2500-3000			1			1				1													3
3000-3500		1	2	1	1	3	2	2	4			1	1										18
3500-4000				1	2		1	3	1	1	3		1	1			1						15
4000-4500					1		1		2	3	1	2	3	2	3	1	1						20
4500-5000					1			1	3		2	1	2	2	4	2	1	1	1				21
5000-5500													1		1		1	1	1				5
5500-6000															1		1			1			3
6000-6500																					1	1	2
Total	1	1	3	2	5	4	4	6	10	5	6	4	8	5	9	3	5	2	2	1	1	1	88

TABLE 49

Correlation surface for amount of eight-months milk production for the first five lactations in the cow's life and the amount of eight-months milk production at three years old for pure-bred Jersey cows.

3 years old	Total production for first five lactations (eight-months periods)																						
	15000-16000	16000-17000	17000-18000	18000-19000	19000-20000	20000-21000	21000-22000	22000-23000	23000-24000	24000-25000	25000-26000	26000-27000	27000-28000	28000-29000	29000-30000	30000-31000	31000-32000	32000-33000	33000-34000	34000-35000	35000-36000	36000-37000	Total
2500-3000									2														2
3000-3500	1	1	2	2	1		1	1	1														10
8500-4000			1		2	1	3	1	1	1	1				1								12
4000-4500					2	3		2	4	1	2												14
4500-5000								1	2	2	2	2	4	2									15
5000-5500								1		1	1	1	2	1	3	1	1						12
5500-6000												1		1	1		1		1				5
6000-6500													2	1	2	1	2		1				9
6500-7000															2	1		1					4
7000-7500																		1		1	1		3
7500-8000																	1					1	2
Total	1	1	3	2	5	4	4	6	10	5	6	4	8	5	9	3	5	2	2	1	1	1	88

TABLE 50

Correlation surface for amount of eight-months milk production for the first five lactations in the cow's life and the amount of eight-months milk production at four years old for pure-bred Jersey cows.

	Total production for first five lactations (eight-month periods)																						
4 years old	15000-16000	16000-17000	17000-18000	18000-19000	19000-20000	20000-21000	21000-22000	22000-23000	23000-24000	24000-25000	25000-26000	26000-27000	27000-28000	28000-29000	29000-30000	30000-31000	31000-32000	32000-33000	33000-34000	34000-35000	35000-36000	36000-37000	Total
3000-3500	1	1		1																			3
3500-4000			1	1	1	1																	4
4000-4500			2		4	3	1		3		1		1										15
4500-5000							1	3	3			1			2								10
5000-5500							2	3	2	5	4	2			2		1						21
5500-6000									2		1	1	5	1	1								11
6000-6500													1	1	3				1				6
6500-7000													1	3	1	2	1	1	1	1			11
7000-7500																1	2	1			1		5
7500-8000																	1						1
8000-8500																						1	1
Total	1	1	3	2	5	4	4	6	10	5	6	4	8	5	9	3	5	2	2	1	1	1	88

TABLE 51

Correlation surface for amount of eight-months milk production for the first five lactations in the cow's life and the amount of eight-months milk production at five years old for pure-bred Jersey cows.

	Total production for first five lactations (eight-months period)																						
5 years old	15000-16000	16000-17000	17000-18000	18000-19000	19000-20000	20000-21000	21000-22000	22000-23000	23000-24000	24000-25000	25000-26000	26000-27000	27000-28000	28000-29000	29000-30000	30000-31000	31000-32000	32000-33000	33000-34000	34000-35000	35000-36000	36000-37000	Total
3000-3500	1		1				1	1															4
3500-4000		1	1	2	2	1																	7
4000-4500			1		1			1	2														5
4500-5000					1	3	3	3	2					1									13
5000-5500					1			1	4	2	2		3		1	1							15
5500-6000									1	2	1	3	1		1	1	1						11
6000-6500									1	1	2		3	2	2	1		1					13
6500-7000											1			1	2		2			1			7
7000-7500												1	1		2		1	1	2		1		9
7500-8000															1							1	2
8000-8500														1			1						2
Total	1	1	3	2	5	4	4	6	10	5	6	4	8	5	9	3	5	2	2	1	1	1	88

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TABLE 52

Correlation surface for amount of eight-months milk production for the first five lactations in the cow's life and the amount of eight-months milk production at six years old for pure-bred Jersey cows.

Total production for first five lactations (eight-months period)

6 years old	15000-16000	16000-17000	17000-18000	18000-19000	19000-20000	20000-21000	21000-22000	22000-23000	23000-24000	24000-25000	25000-26000	26000-27000	27000-28000	28000-29000	29000-30000	30000-31000	31000-32000	32000-33000	33000-34000	34000-35000	35000-36000	36000-37000	Total
3000-3500	1		2																				3
3500-4000		1	1		3	1	1		1														8
4000-4500				1	1			1															3
4500-5000				1	1	2	2	1	3	2			1										13
5000-5500						1	1	3		1	2	1	1	2	1		1						14
5500-6000								1	5	1	1	1	1		2								12
6000-6500											2	2	3	1	3	1	1						13
6500-7000									1	1	1			1		1		1				1	7
7000-7500													1	1	2	1	1				1		7
7500-8000													1		1		2	1		1			6
8000-8500																			1				1
8500-9000																							0
9000-10000																			1				1
Total	1	1	3	2	5	4	4	6	10	5	6	4	8	5	9	3	5	2	2	1	1	1	88

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TABLE 53

Lactations correlated	Correlation coefficients	Average correlation coefficients		Correlation coefficients for given lactation and total of five lactations minus the given lactation	
1 and 2	+0.5991 ± .0461	1st lactation		1st and	
1 and 3	.6007 ± .0459	and 2nd, 3rd, 4th, 5th	0.5676 ± .0486	2 + 3 + 4 + 5	0.6475 ± .0418
1 and 4	.5671 ± .0488	2nd lactation		2nd and	
1 and 5	.5033 ± .0537	and 1st, 3rd, 4th, 5th	.6284 ± .0430	1 + 3 + 4 + 5	.7352 ± .0331
2 and 3	.7785 ± .0284	3rd lactation		3rd and	
2 and 4	.5851 ± .0473	and 1st, 2nd, 4th, 5th	.6445 ± .0416	1 + 2 + 4 + 5	.7768 ± .0285
2 and 5	.5509 ± .0501	4th lactation		4th and	
3 and 4	.6213 ± .0442	and 1st, 2nd, 3rd, 5th	.6098 ± .0451	1 + 2 + 3 + 5	.7075 ± .0359
3 and 5	.5775 ± .0480	5th lactation		5th and	
4 and 5	.6657 ± .0400	and 1st, 2nd, 3rd, 4th	.5744 ± .0480	1 + 2 + 3 + 4	.6924 ± .0375

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the milk production of two-year-olds with the total production of the next four lactations and compare these correlations with those for the two-year-olds correlated separately with each of the other lactations we will have a measure of the action of environment in affecting milk production of the cow as against the relatively steady environment over a longer period. The data of table 47 furnish the constants for the necessary calculations of the long-time lactation periods. The calculations for the short time involve the calculation of twelve correlations. These data are shown in table 53.

The correlation coefficients of the given lactation with the total of the first five minus the given lactation are in general about 0.1 higher than the mean correlation coefficient as found by averaging the correlations of the separate lactation with the given one. The consistency of the difference rather than the amount leads to the conclusion that this difference while slight is biologically significant. Such being the case the environment may be roughly said to effect a lowering of the correlation coefficient which on an average amounts to from one-seventh to one-eighth the correlation coefficient of the average environment under which these cows live.

It is impossible to get any accurate means of measuring the relative influence of the environmental conditions of the younger years. From the history of the herd, the standard conditions for all the young stock and the similarity of the feeding, etc., it seems doubtful if such environmental differences as do exist in the calf period are of as great consequence to the subsequent milk production as are such differences in the actual lactation cycle.

These results are in conformity with those of GALTON (1895) for widely different material on the differential action of "nurture and nature" in favoring the much greater influence of nature over nurture in the somatic expression of a character. The intensity with which a given cow maintains a given plane of milk production throughout life indicates a fair degree of precision in the mechanical operation of the inherited factors which appear in large measure to govern the amount of milk produced.

The comparison of the accuracy with which this control mechanism works with that of similar secretions in other species has a good deal of direct bearing on the subject. The only data available are for the egg of the domestic fowl. This egg, like milk, is composed of a number of substances going to make up the whole. It differs from milk in being recorded in number of eggs produced and not in pounds. The eggs are further produced in clutches, each clutch extending over a short time in the year. These clutches are recurrent and might be considered to parallel the successive lactation cycles. Egg production differs from milk production in a further vital particular. The stimulation of the secretion in an egg depends on the passage down the oviduct of an egg yolk or other foreign body. The amount of egg albumen, etc., secreted depends on the number of egg yolks released from the ovary and not on any innate property of albumen secretion as does the secretion of the parts to form milk. Remembering these differences it is still of a good deal of interest to compare the correlation which may exist between successive periods of egg production and the total number produced in a given period and the correlations for successive periods of milk production and the production for the first five lactations as derived in table 47.

Correlations obtained by HARRIS and BLAKESLEE (1918) for the successive monthly egg productions of White Leghorn pullets with their first annual production, range from +0.372±.030 to 0.695±.018 with an average of 0.556. The correlations of the successive yearly productions and the total of the first five lactations shown in table 47 range from +0.7416±.0323 to 0.8613±.0186. The difference between these correlations is striking. In the proper calculation of this difference it must be remembered that the correlations for egg production is for the twelve-month period where the production for the individual-month correlation contributes only one-twelfth to the total annual egg production while that for the milk production contributes about one-fifth to the total production of the five lactations. The correlation would therefore be expected slightly larger for the milk production. This increased size of the milk correlations over the egg correlations is very small relative to the whole difference, as the confirmatory results of table 38 (page 159) show. The correlations for the milk production of the successive lactations, therefore, represent in concrete terms that a greater reliance may be placed in the milk records of one lactation as measuring the cow's capacity than can be placed in the monthly egg records as measuring the hen's capacity for annual production. Such being the case, if we generalize this conclusion in its ultimate terms, the causative mechanism behind milk production works with greater fineness and precision than does the mechanism for egg production. Since this mechanism seems in its broadest sense to be of hereditary origin in the two cases, it follows that in the cow this hereditary complex is less influenced in its action by environment than is the action of the material stuffs of the fowl for egg production.

PRACTICAL ASPECT OF THE CORRELATIONS FOR MILK PRODUCTION OF ONE LACTATION RECORD WITH ANOTHER LACTATION RECORD

The constants deduced in table 47 have a good deal of practical value to the dairyman and to the student of farm management. To illustrate, suppose a herd which has 1000 two-year-olds which have just completed their first lactation, is chosen at random. The manager of this herd will wish to know what animals to save for future lactations and the student of farm management desires to know what may be expected for the total production of the animals to enable him to determine the plane to which the herd should be culled for the greatest profit to the owner. The equations derived from table 4 on page 120 allow the determination of the distribution of the cows as to their eight-months yield of milk if the one thousand two-year-old cows are drawn at random from a population similar to that of the Jerseys of these data. The equation for this curve may be repeated for sake of convenience of those who desire to check the results.

$$y = 71.7407 \left(1 - \frac{x^2}{16{,}291{,}354}\right)^{10.648}$$

From the actual eight-months milk production of table 47 the equation to determine the sum of the expected lactation yields of the first five lactations is given by

$$Y_T = 7671.9 + 4.3139 y_2$$

where Y_T is the total expected yield of the five lactations and y_2 is the actual yield of the two-year-old cow.

Perhaps one of the easiest ways to table the necessary information for its readiest uses is to have the summation of both the number of cows and their expected total production. This summation to be from both ends of the range of milk produced. The data for such a comparison are given in table 54.

If all the cows in this herd are kept to their sixth lactation the average production for each cow for the five lactations would be 25,070 pounds of milk. Noting the summed number of cows in column four if the 217 poorest producers are culled from the herd the cows left in the herd will produce on an average 26,408 pounds or on an average 5,389 pounds more milk than the culled cows. Again if the dairyman decides to cull out 543 of the thousand animals the production of the 457 remaining will be 28,192 pounds or 5,747 pounds more than the culled cows on the average for the summed productions for the five lactations.

Actual mil ected five-lactation yield for any division of division.†

Actual mil production	med er of highest west ction	Expected summed five-lactation yield for cows in herd highest to lowest	Expected production per cow lowest to highest	Expected production per cow highest to lowest
1375 to 162	00	25,069,737	14,143	25,070*
1625 to 187	99	25,050,000	14,822	25,076
1875 to 212	97	25,028,382	15,794	25,098
2125 to 237	92	24,941,016	16,734	25,146
2375 to 262	80	24,734,564	17,649	25,240
2625 to 287	57	24,316,340	18,538	25,401
2875 to 312	19	23,571,666	19,400	25,644
3125 to 337	62	22,387,833	20,229	25,979
3375 to 362	83	20,688,056	21,019	26,408
3625 to 387	86	18,465,437	21,761	26,926
3875 to 412	74	15,803,438	22,445	27,524
4125 to 437	57	12,874,227	23,063	28,192
4375 to 462	43	9,908,266	23,604	28,920
4625 to 487	41	7,146,761	24,056	29,698
4875 to 512	57	4,788,135	24,416	30,517
5125 to 537	04	2,948,571	24,683	31,371
5375 to 562	51	1,647,557	24,864	32,254
5625 to 587	25	821,787	24,974	33,163
5875 to 612	1	358,346	25,032	34,096
6125 to 637	4	132,856	25,057	35,054
6375 to 662	1	40,385	25,070*	36,058

---

* It will hat of table 49. The reason for this is that table 54 is roduction of 4032.89 (see table 4) while table 49 is based 159.1. The corresponding total five-lactation expected re

† All calc es, otherwise the results were found to be only appro rest whole number.

As the records dealt with in this paper are all for the first eight months of lactation it follows that most cows will extend beyond this limit and produce more milk. In using this table to determine what cows may be kept profitably, this fact should be kept in mind. If the dairyman has determined the complete cost of producing his milk a knowledge of the price he receives for it will allow him to determine at once from column three what cows he should keep in the herd.

THE RELATIVE CONTRIBUTION OF ONE YEAR'S MILK PRODUCTION TO THE TOTAL PRODUCTION OVER THE FIRST FIVE LACTATIONS

A little thought on the subject will convince one that even though there is a substantial correlation between the lactation records of the individual lactation and the total production over several lactations it by no means follows that the individual cows necessarily contribute the same relative amounts of milk to the individual lactations and to the total of the five-lactation groups. The problem may be quantitatively analyzed by the determination of the correlation between the total of the five lactations of the individual cows and the deviation of the pounds of milk produced by them in the single lactations from their probable value. The calculations of this correlation may be most easily made by the method of Harris (1909, 1918). The formula for the correlations is given by the equation

$$r_{xz} = \frac{r_{xy} - v_x/v_y}{\sqrt{1 - r_{xy}^2 + (r_{xy} - v_x/v_y)^2}}$$

where the y's represent the individual lactation records and the x's the totals for the five lactations. The five constants for these are tabled in table 55.

Table 55

Correlation between the total milk production of the five-lactation records and the deviation of the individual records from their probable values.

Age when correlation records were made	Correlation	Correlation ÷ probable error
2 years and total	—0.2897 ± .0659	4.39
3 years and total	+ .1905 ± .0693	2.75
4 years and total	— .0667 ± .0716	.93
5 years and total	— .0240 ± .0719	.33
6 years and total	+ .0087 ± .0719	.12

These correlations of table 55 change their sign three times, two of them being plus and three minus; their magnitudes range between $+0.1905 \pm .0693$ and $-0.2897 \pm .0659$. As adjudged by their probable errors only two of the correlations can be considered significant. Taken at their face value these coefficients would seem to indicate that the highest producers at two years contribute relatively less to the total production over several lactations than was their expected share and that these same high producers contribute relatively more in their second lactation to this total than was to be expected of them. In the other lactations each individual of a given grade contributed approximately its proportionate expected share to the total production. These relations of the deviations from their probable yields of the first and second lactations are hard to account for in terms of the known physiology of the mammary gland. The parallel already indicated of the logarithmic nature of growth and milk production with age seems to point to a possible causal relation between growth of the mammary gland and the quantity of its secretion. Should this prove true the problem of this differential relative contribution of the high producers at two years old and at three becomes a problem of the relative duration of growth of the mammary gland in the high, medium and low producers and not a cyclic action of different sets of factors for milk production itself. This means that the factors which govern milk production have their regulating power maintained in the same relative strength throughout the life of the cow with the exclusion of any group of factors acting within this life time for any short period.

SUMMARY

The purpose of this paper is to present quantitative data, along with the biometrical analysis of the same, on the normal variations and correlations of milk production during the cow's life. The necessity of such data and analyses is well recognized now, in physiological and genetic investigations of a quantitative subject. The economic value of milk gives this investigation a significant relation to improvement in yield and in the selection of cows to remain within the herd. The materials used for this study are peculiarly suited to the ends in view. They are a strictly homogeneous group of milk records of normal pure-bred Jersey cows maintained under the conditions of a large farm, managed by a well-trained man thoroughly versed in his business. While the conclusions derived from this study are stated in general form, it is not presumed that the conclusions have more generality than the data on which they are based. It is altogether probable however that the conclusions drawn do apply to most of the other dairy breeds of cattle.

The conclusions of most moment may be summarily stated as in the following paragraphs. Unless otherwise stated, the conclusions apply to the pure-bred Jersey herd the data for which are given in this paper.

The frequency polygons for milk production are only slightly unsymmetrical. The range of variation is large, one cow producing less than 1500 pounds in her eight-months lactation period, another cow producing more than 10,000 pounds for her eight-months lactation period. The height of the modal ordinate for the frequency polygons is greatest in the earlier and later years of the cow's life. Only one mode is evident in any of the histograms. From this it appears likely that these curves are unimodal curves. Too much stress should not be laid on this however since certain of the distributions appear quite flat-topped.

The analytical constants show that the eight-months-milk-production curves are of the PEARSON types I, II and IV. The skewness of these curves is all in the positive direction and rather small in amount. This small size of the skewness is of considerable interest to those investigators who deal with advanced-registry data since it would indicate that these data can with but small error be fitted by normal curves to determine certain of their needed constants.

The correlation coefficient between age at the commencement of test and milk production in comparison with the correlation ratio for the same variables shows that the regression of milk production on age is far from linear. This regression curve when analyzed turns out to be a logarithmic function. Attention is called to the fact that this same kind of logarithmic function also describes the relation of growth to age. This paralleling of the two functions may indicate that a causal relation exists between them such that increased milk production chiefly depends on increase in size of the mammary gland caused by growth.

The standard deviations of milk production progressing from the heifer to the aged cow are described by a cubic parabola. This regular change of the standard deviation of milk production groups shows that the curve formed by them falls into the heteroscedastic, nomic class as defined by PEARSON.

Correlation tables for milk production of the different age groups are presented.

The means derived from these tables remain, within the limits of random sampling, the same for any given age at test irrespective of the age at which the same cows were subsequently tested.

The standard deviations for the various age groups also remain at the same value within the limits of sampling irrespective of the age at which a subsequent test may be made.

As would be expected from their derivation and as actually checks out to be the case the coefficients of variation do not change within the age group for the cows which are retained in the herd for longer or shorter periods. These facts show that the cows which were kept in the herd as two-year-olds were all equally likely to be kept in the herd until they became aged cows as far as the milk production of these two-year-olds was concerned. Generalizing this statement cows which were kept in the herd at any given age were all equally likely to be kept in the herd at any other age as far as the milk record of any of those cows at the given age was concerned. This point removes any possible objection to the accuracy of the correlations for the milk productions at the various ages at least so far as concerns the cows remaining in the herd on the basis of their milk production being high.

The correlation coefficients for the milk production of one lactation with that of another lactation at another age range from +0.2144 ±.0919 to +0.7306±.0284. The signs of these correlation coefficients are all plus. The numerical values are large enough to be significant. Each set of correlation coefficients for the milk production at a given age with the milk productions at other ages in the cow's life deviate only slightly from a line which is approximately linear, taking a nearly constant value for each age. The mean value for these correlation coefficients of milk production of one lactation at any age with the milk production of another lactation at another age is +0.5352.

This mean value of the coefficient of correlation seems to be generally applicable to milk production for on somewhat similar data GAVIN finds results which are comparable to these.

These correlation coefficients are high for this kind of data as may be seen from the fact that correlation coefficients of similar nature for egg production range lower and have a lower mean +0.4458 than do these correlation coefficients for milk production.

The magnitude of these correlation coefficients in comparison to those on egg production put in terms of the physiology of the two sets of glands, mammary and ovary, means that the mammary gland works with more mechanical precision than does the ovary.

The linearity of all the regressions is established by the calculation of the correlation ratios and their comparison with the corresponding correlation coefficients.

From the means, standard deviations and correlation coefficients are formed the straight-line equations for the prediction of the amount of milk production of any given lactation at any age from these known variables at another age. These equations are given in table 43. Examination of these equations seems to show that most stress is laid on the milk production at two years old by the coefficients, indicating that the milk production at this age represents more accurately than any other the future possibilities of the cow. Similar analysis reveals the fact that correction of the milk records to about the age of maximum productivity of the cow gives the nearest approach to the correct milk production for that cow.

The correlation coefficients of milk production at a given age with the total milk production of two lactations are presented in table 46. These correlations prove to be very high, ranging from +.7324±.0286 to +0.9438±.0116. Such high values make it appear that the milk production of one lactation is the determining factor for total milk production of two lactations when the given lactation is one of the components.

This conclusion is confirmed by a study of a smaller series of data where the first five lactation records are known, the first commencing at two years old and the other four commencing approximately a year apart. These correlation coefficients for milk production of a lactation at a given age with the total milk production over the five lactations range from +0.7416±.0323 to +0.8613±.0186. Each regression is linear as is shown by a comparison of the correlation coefficients and correlation ratios.

A means of measuring the effect of environmental changes on the coefficient of correlation of the milk production of a lactation at a given age with the milk production of a lactation at another age is given. This measure depends on the difference in the correlation coefficients of the milk production of a given age correlated with the milk productions of several successive lactations and the correlation of the milk production of the given lactation and the total milk production of the other lactations. In the first case the environmental changes of the different lactations as they affect milk production make themselves felt in the correlation coefficients; in the second case the use of the total milk production gives a mean of these environmental effects. The outcome of this study shows the environmental changes reduce the correlation one-ninth to one-eighth of what it would be under the conditions of an average environment. This result makes it clear that for these Jerseys heredity plays a greater part in their milk production from lactation to lactation than environment.

The correlation coefficients for the milk production of one lactation with that over several lactations, furnishing as it does the basis for the regression formula by which milk production over long periods may be predicted from shorter ones, is of the greatest practical importance in culling the herd of the mediocre animals. The use of these coefficients is illustrated in table 54.

The relative contribution of the different lactations to the total milk production over a long period shows that in the first lactation the high-producing cows do not produce quite as much relatively as they would be expected to produce. In the second lactation these same high-producing cows produce relatively more than they would be expected to produce. Thereafter the contribution of any class is at random. The significance of the relative contribution of the first two years is only slight. In view of these facts the conclusion may be drawn, that the factors which govern milk production have their regulatory power maintained in the same relative strength throughout the life of the cow to the exclusion of any group of factors acting within this life for any short period.

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A GENETICAL STUDY OF FLOWER FORM AND FLOWER COLOR IN *PHLOX DRUMMONDII*

JAMES P. KELLY
Department of Botany, Pennsylvania State College, State College, Pennsylvania

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INTRODUCTION

Of the thousands of species of plants known to science, surely of not more than a half dozen can the student of heredity claim to have a fair knowledge. Hereditary study of the modern Mendelian sort is dependent on "the presence of differential unit characters" (SHULL 1910), and it is somewhat surprising that in the case of *Phlox Drummondii* with its large number of strains possessing marked differentiating characters no extensive analysis has yet been presented. The author has already discussed the variation in this species (KELLY 1915), having pointed out that there are forms that are tall, some that are half-dwarf, and some that are dwarf; some are large-flowered and some small-flowered; the inflorescence may be loose and spreading or rounded and dense; the flower may be single, half-double, or fully double; the fully opened flower may be salver-shaped or funnel-shaped; the colors have a wide range from white and cream through pinks, lilacs, roses, purples and reds, to almost black; the flowers may have dark centers or eyes, or white centers; the corolla lobes may be wide and markedly overlapping or narrow; the corolla lobes may be entire or variously laciniated. One laciniate type (var. *fimbriata*) was many years ago reported to be inconstant (SPRENGER 1888). The 1914 catalogue of HAAGE and SCHMIDT, a large German seed-firm, offered for sale 173 varieties within this single species. This wealth of variation is known to have occurred subsequent to 1835, and without the aid, as far as the evidence goes, of crossing outside of the species.

Only one bit of experimental work on the genetics of *Phlox Drummondii* has thus far appeared, namely, a paper by A. W. GILBERT (1915), who carried three crosses through the F_2 generation, and assumed five factors to explain his observations: A dark-eye factor, a blue factor, a red factor, an intensifying factor, and a cream factor. The question of factor interactions was raised by his work, as he expressly made cream dependent on the presence of the dark-eye factor (presumably the same dark-eye factor that is necessary for the production of an anthocyanin eye); and his intensifying factor functioned visibly only when the red was present he also tacitly assumed (as the factorial formulae in his tables prove) an important interaction effect between the blue and red factors; thus in one place it appears that the blue factor alone gave rise to a lilac-colored flower, the red alone to a pink, but these two factors together were supposed to give a deep(full-)-colored carmine type. GILBERT carried none of his experiments beyond the F_2 generation and confirmation of his formulae is therefore lacking. A preponderance of white-flowered plants following one of his crossings was "unaccounted for."

The present paper is offered as a further contribution to the genetical analysis of the species. It is based on experimental data secured chiefly while pursuing graduate work in the Department of Biology of PRINCETON UNIVERSITY[1]; the author is indebted to Dr. G. H. SHULL, under whose auspices the work was carried on, for suggestions and an encouraging interest in the progress of the work; and to Dr. E. N. HARVEY for enlightenment on the chemical aspects of the color problems. Acknowledgments are also due to Prof. R. A. EMERSON, of CORNELL UNIVERSITY, for valuable criticisms. The paper will concern itself first with certain variations in the form of the flower; then with an analysis of color conditions in the flower.

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[1] Much necessary preliminary work had been done at the PENNSYLVANIA STATE COLLEGE.

GENETICS OF FLOWER FORM

The "Star Phloxes"

In the spring of 1914 the writer sowed seeds of what the seedsman called "Star Phlox" and was impressed by the decided variation exhibited in the flowers when the plants came to maturity; one plant showed the flowers entire-edged and gave similar offspring, others bore flowers that were variously fringed or toothed. A study of the character and history of these variations was undertaken.

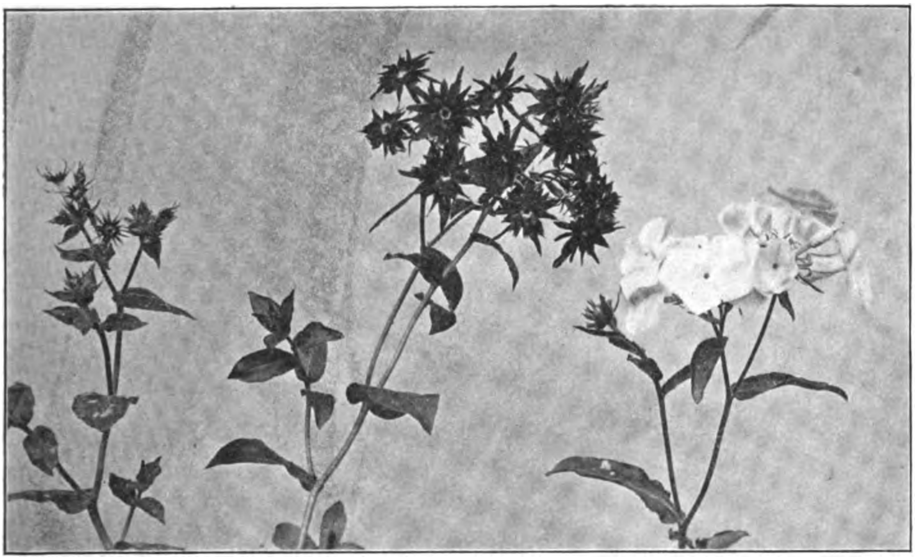


FIGURE 1.—To the left, a *cuspidata* plant (type L). To the right, a plant with entire-edged flowers (type A). In the center, the F_1 hybrid of A and L (this hybrid is of the *fimbriata* type). *Cuspidata* and *fimbriata* are "star phloxes."

As elsewhere pointed out (KELLY 1915) *Phlox Drummondii,* of which species the "Star Phloxes" are a variety or varieties, was first introduced to cultivation in 1835 and the original phloxes had entire-margined flowers. A perusal of horticultural literature published since 1835 shows that the varieties with fringed or toothed corolla segments, i.e., the "Star Phloxes," were unknown till about 1887 when they seem to have created quite a stir in German horticultural circles; in that year the firm of MARTIN GRASHOFF was awarded a certificate of merit for introducing two novelties, both of which are grouped together under the popular name of "Star Phloxes." The flowers of both novelties had fringed or incised edges. WITTMACK (1888) named these *P. Drummondii* var. *fimbriata* and *P. Drummondii* var. *cuspidata*. On a cursory examination *cuspidata* can be distinguished from *fimbriata* by the smaller body of the flower, meaning by *body* the non-incised residuum of the

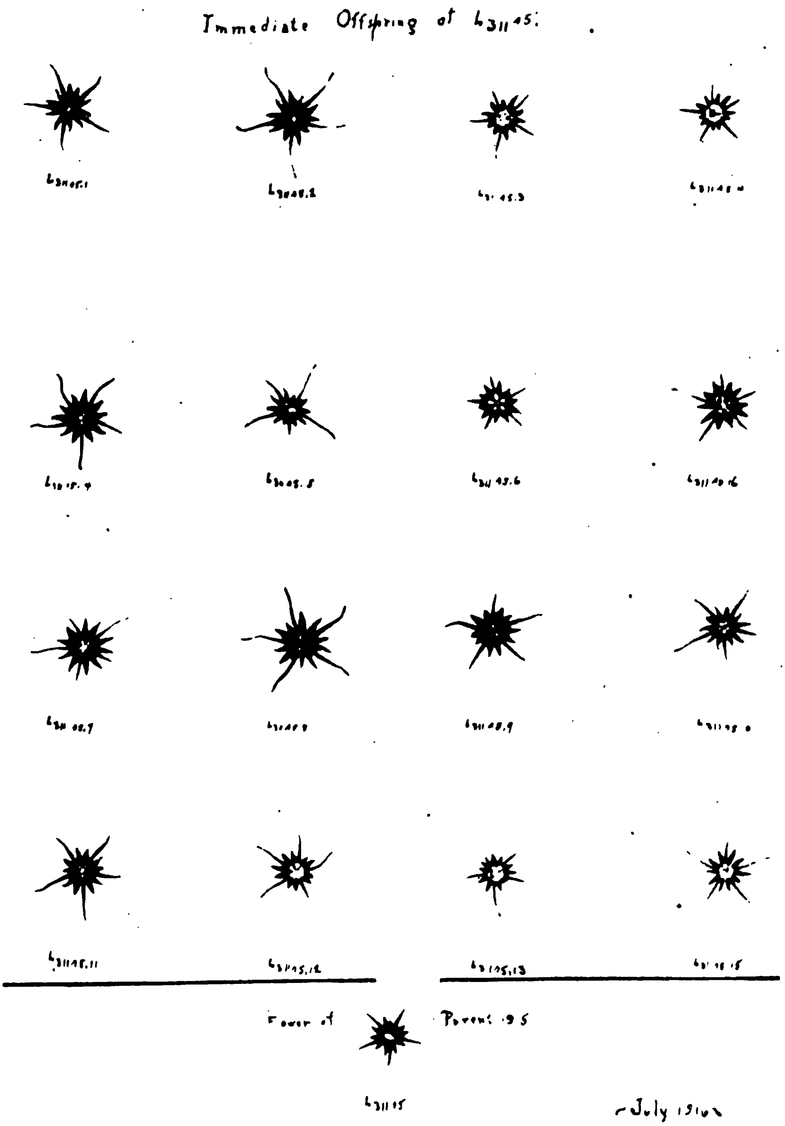


FIGURE 2.—The 16 flowers above represent a family of *cuspidata* plants; each of the 16 flowers is from a separate plant. Below, a flower from the parent of this family.

flower limb immediately surrounding the throat. Figures 2 and 5 show the contrast at a glance. This body, of course, will be in the five sections or lobes characterizing Phlox flowers of every kind; in *fimbriata* and *cuspidata,* however, each lobe bears three teeth, the middle tooth in *fimbriata* being one to four times the length of the side teeth, while in *cuspidata* it is perhaps six times the length of the side teeth.

Complete or at least a high degree of constancy through seed should ordinarily characterize a *variety* of an annual plant like *Phlox Drummondii.* A voice was soon raised against the listing of *fimbriata* as a good variety. C. SPRENGER (1888) grew plants, evidently from commercial seed, in his garden near Naples in 1888 and found but 19 *fimbriata* plants in a total of 94; he deemed this insufficient stability to warrant *fimbriata* being given varietal rank. SPRENGER assented, however, to the listing of *cuspidata* as a good variety. That these objections to *fimbriata* as a variety proved fruitless is seen from BRAND's (1907) treatment of the Polemoniaceae in "Das Pflanzenreich." BRAND mentions both var. *fimbriata* and var. *cuspidata* in his treatment of the types of *Phlox Drummondii* and he moreover gives himself as authority for having once raised both types to full specific rank.

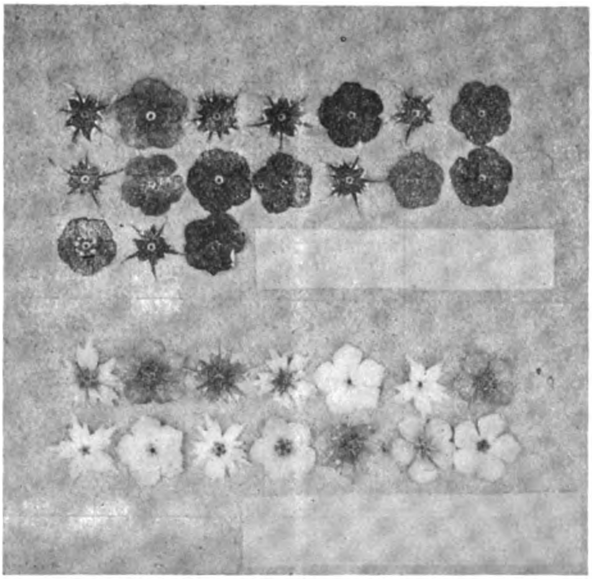


FIGURE 3.—Two families derived from back-crossing of *fimbriata* to entire. Each flower represents a single plant.

The experimental results of the writer have made clear the interrelationships of *cuspidata, fimbriata,* and the entire-edged flowers. One *cuspidata* plant of 1915 ($L_{311.5}$) was the ancestor of all the *cuspidatas* used in these experiments; there have descended directly from it, in spite of the difficulties of securing selfed seed, a total of 41 plants, all, without exception, of *cuspidata* type. Four families descended from segregate *cuspidata* plants of known hybrid extraction were also unexceptionally *cuspidata.* As far as tested, then, *cuspidata* is constant for its peculiar flower form. Figure 2 shows a flower of $L_{311.5}$ and one from each of its 16 progeny. Plants with floral periphery entire have been found to give, on inbreeding, only progeny with entire margins.

The crossing of *cuspidata* plants with those whose flowers were entire has been made in different seasons with results that have been always the same,—the F_1 hybrids have been of the *fimbriata* type. This particular hybridization has been made 12 times giving rise to a total progeny of 94 F_1 plants, all of which bore *fimbriata* flowers (see figure 1 for the parental and F_1 types).

On back-crossing *fimbriata* with the entire-edged type there appear only *fimbriata* and entire in about equal numbers. Several back-crosses of this kind have been made, yielding a total of 121 progeny, of which 52 were *fimbriata* and 69 bore flowers that were entire (see figure 3 and table 1).

TABLE 1

Tabular presentation of back-crosses of fimbriata to entire.

Back-cross	Immediate progeny of back-cross			Total
	Cuspidata	*Fimbriata*	Entire	
Entire × $(AL)_{307}$	0	7	10	17
$(AL)_{307}$ × entire	0	18	23	41
$(AL)_{308}$ × entire	0	6	6	12
Entire × $(LA)_{302}$	0	13	19	32
Entire × $(GL)_{12}$	0	1	4	5
Entire × *fimbriata* of commercial origin	0	7	7	14
Totals	0	52	69	121

Considerable data have accumulated concerning the character of the progeny of self-fertilized *fimbriata* plants of known origin. The total number of offspring thus produced through several summers has been

706. Of these 179 bore entire flowers, 353 were *fimbriatas*, and 174 were *cuspidatas* (see table 2 and figures 6 and 7).

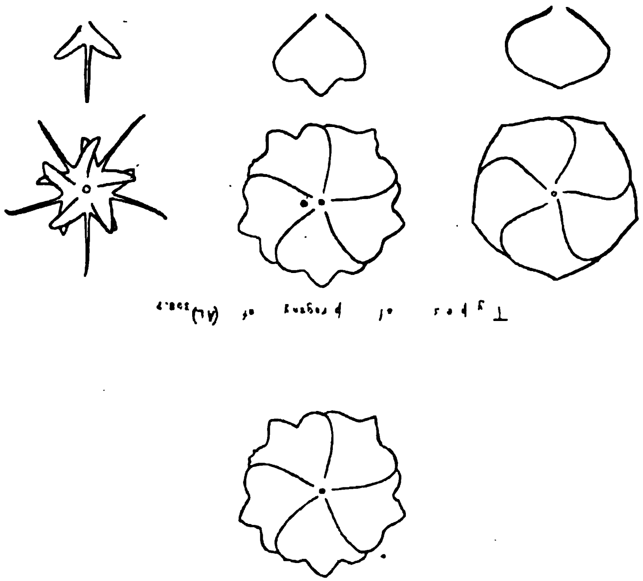


FIGURE 4.—Above, diagram of a shallowly incised *fimbriata* flower. The three flowers on the second line are diagrams of all the flower types occurring among the progeny (by selfing) of a shallowly incised *fimbriata* plant. The lowermost line gives diagrams of single corolla segments of each of the types. Compare with diagrams in figure 5 and note that shallowly laciniated plants are associated with entire-lobed sibs that are wide-lobed, and with cuspidate sibs of a peculiar type.

The experimental results set forth demonstrate very clearly that in *Pholox Drummondii* the type with entire-margined flowers differs from the *cuspidata* type by a single pair of genes; that there is a lack of dominance of the differentiating gene, which results in the production of a distinct heterozygous type called *fimbriata*; and that this F_1 form, *fimbriata*, can give but 50 percent of *fimbriata* offspring (25 percent being *cuspidata* and 25 percent entire) in accordance with simple Mendelian expectations. The bearing of this conclusion on the taxonomic position assigned by BRAND to *fimbriata* is obvious; *fimbriata* should not have specific rank; even varietal rank should be denied it; it should be designated as an ever-splitting hybrid form like the Blue Andalusian fowl.

Segregates from *fimbriata* plants have been tested, with results which support the general conclusion reached in the preceding paragraph.

TABLE 2
Composition of progenies of self-fertilized fimbriata.

Pedigree number	Entire	*Fimbriata*	*Cuspidata*	Total
(LJ)$_{1}$	2	3	1	6
(LJ)$_{106}$	1	1	3	5
(GL)$_{5}$	2	0	3	5
(GL)$_{12}$	5	8	6	19
(GL)$_{12.5}$	3	9	5	17
(GL)$_{12.6}$	3	3	1	7
(AL)$_{331}$	13	43	14	70
(AL)$_{304}$	6	9	4	19
(AL)$_{306}$	3	11	5	19
(LA)$_{302}$	19	25	12	56
(AL)$_{307}$	4	13	4	21
(AL)$_{331.63}$	19	29	16	64
[C(AL)]$_{1}$	16	43	23	82
[B(LA)]$_{1}$	11	26	10	47
(AL)$_{331.5}$	1	2	2	5
(AL)$_{306.7}$	13	21	11	45
(AL)$_{101}$	30	51	28	109
[A(LA)]$_{1}$	8	14	2	24
(AL)$_{331.54}$	5	9	6	20
(AL)$_{331.29}$	13	27	13	53
(AL)$_{102}$	2	6	5	13
Totals	179	353	174	706

Eight entire-flowered segregates gave families averaging over 24 offspring each, every one of which bore entire-margined flowers (see figure 9). Four *cuspidata* segregates produced families averaging over 41 progeny each, every one of which was *cuspidata;* no entire flowers occurred nor any that were *fimbriata* (see figure 8).

A glance at the culture conveys the impression that the fimbriate and cuspidate flower shapes are independent of color; they can be transferred from one color type to another. A narrow white or whitish band found invariably bounding the outer edge of the *cuspidata* and *fimbriata* flowers, however, has never been found shifted to segregates with entire-edged flowers.

Concerning the variability of the types it should be said that it is to be expected in the case of any series of multiple structures like the collection of flowers on a plant that variations will occur. The flowers on a *fimbriata* plant may show fairly noticeable variation but it is not difficult to recognize a typical flower for each plant and then easily to separate the fimbriate from the cuspidate types, or types of fimbriates from one another.

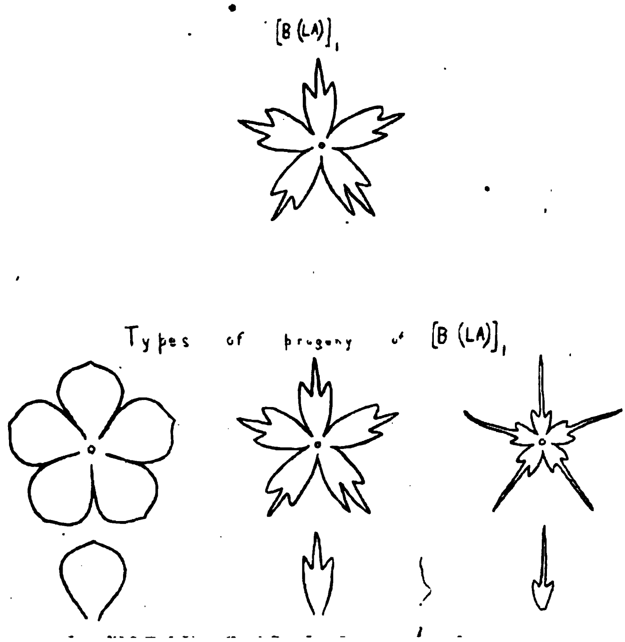


FIGURE 5.—Above is a diagram of a flower of a sharply and rather deeply incised *fimbriata* parent. The three flowers on the second line are diagrams of all the flower types appearing among its offspring. The lowermost line gives diagrams of single corolla lobes of these types. Compare with figure 4.

Some of the differences in the case of the *fimbriatas* are obviously genetic. In one F$_3$ family descended from a shallowly fringed *fimbriata* parent, designated (AL)$_{308.7}$, there were 11 *cuspidatas*, 21 *fimbriatas*, and 13 entires; all of the 21 *fimbriatas* were shallowly fringed like the parent, as shown in figures 4 and 6. The non-incised central residuum of the flower of these *fimbriatas* was nearly as extensive as the blade in the flowers of their entire sibs; the teeth were very short. An examination of the rest of the family revealed that the shallow type of *fimbriata* was associated with *cuspidata* sibs of a unique kind (see the 11 plants represented to the left in figure 6); the lateral teeth on each corolla lobe were longer and heavier than in the (typical) *cuspidata* first described, and project out more or less tangentially rather than radially; this odd type of *cuspidata* on another occasion was found to be true-breeding. Also accompanying the shallow *fimbriata* was a breadth of corolla lobe in the entire sibs that was so great that they decidedly overlapped.

Another F$_3$ family, descended from a *fimbriata* plant designated [B(LA)]$_1$, made interesting comparison with the foregoing one. It com-

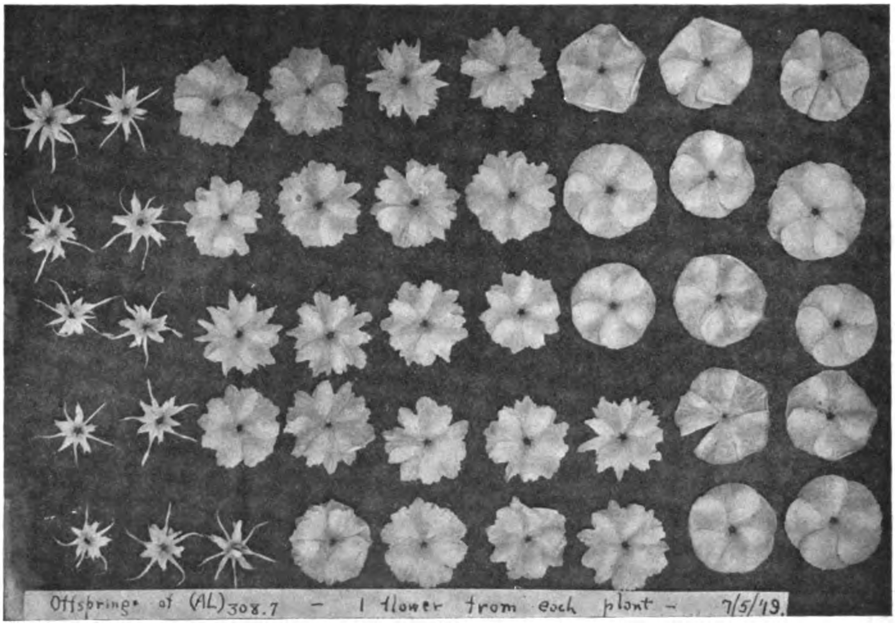

Offsprings of (AL)308.7 — 1 flower from each plant — 7/5/19.

Figure 6.—A single family descended from a *fimbriata* parent whose flowers were shallowly incised like the fimbriates here shown. Note that the sibs of shallow *fimbriatas* are wide-lobed entires and the (new) type of *cuspidata* whose side teeth extend more or less tangentially. One flower from each plant of the family.

prised 10 *cuspidata*, 26 *fimbriatas*, and 11 entires (see figures 5 and 7). The *fimbriatas* throughout were more deeply incised and resembled their parent; the middle tooth of each corolla lobe was longer in comparison to its adjoining side teeth than was the case in the shallow *fimbriatas* discussed in the preceding paragraph. Occurring as sibs with this more incised *fimbriata* was the typical *cuspidata* whose side teeth were short and extending radially, and a narrow-lobed type of entire.

The two *fimbriata* types just described evidently differed because of some modifying factor or factors that are non-specific; these affect the condition both of the *cuspidata* and of the entire. Other families show a mixture of types.

An extreme and unusual variation arose on a portion of a single *fimbriata* plant in 1918; a few of the flowers had entire margins, these being confined to a small part of the plant which was otherwise *fimbriata*. A bud sport was suspected and seed from the entire-flowered branch was separately sown in 1919. It gave rise to 2 entire-flowered plants, 6 *fimbriata* and 3 *cuspidata* plants, and none of the *fimbriatas* again bore

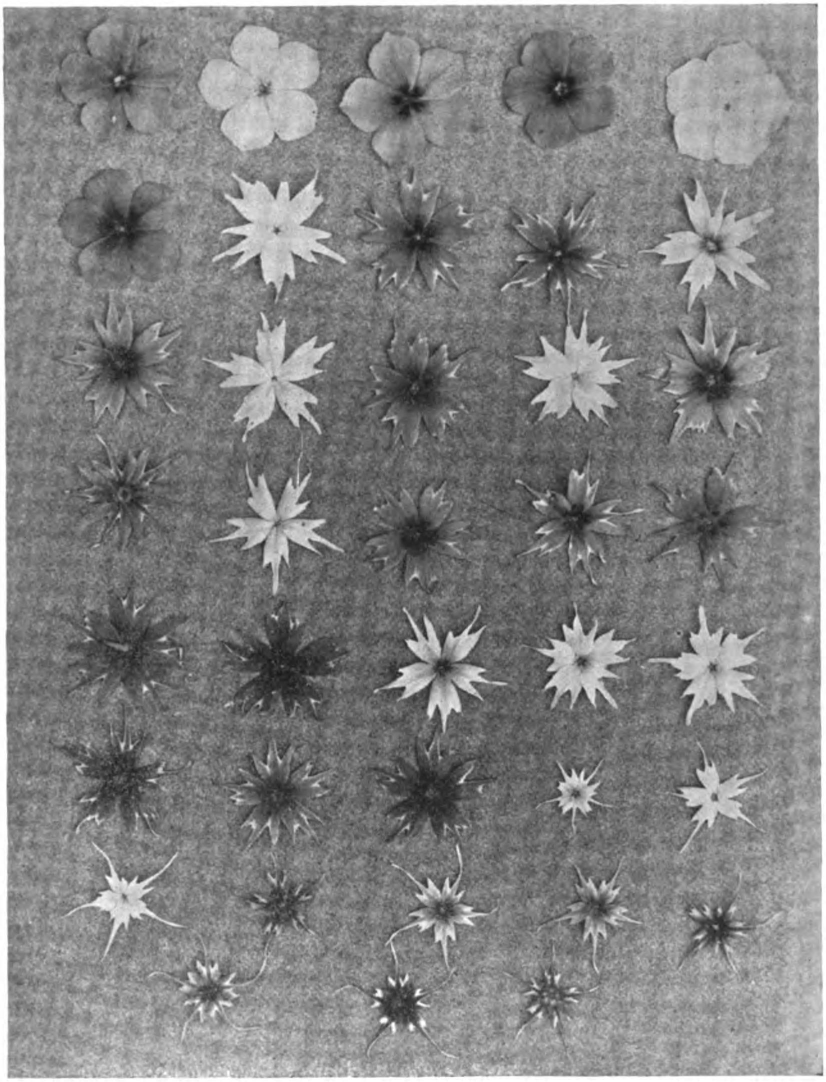


FIGURE 7.—All of the flowers of this figure represent a single family which was derived from a *fimbriata* parent of type diagrammed at top of figure 5. Each flower was from a separate plant and was typical of the plant. Attention is directed to the narrow-lobed condition of the entire flowers and to the types of the ***fimbriata*** and ***cuspidata*** sibs associated with narrow-lobed entires. (Nine sibs of these plants—5 entires and 4 *fimbriatas*—bloomed later and were not included in the photograph.)

entire flowers. The 1919 data might be taken to indicate that the variation to the entire condition was non-genetic, that it was merely a remarkably wide fluctuation. We should keep in mind, however, that the epidermis in the entire-flowered branch might have become, through a mutation or a somatic segregation in the dermatogen, genetically distinct from the other floral tissues. A constitutional change that was so localized would not be transmitted by the germ-cells of the flower since these are hypodermal in origin. The epidermis leaves no progeny, in other words, and its hereditary composition cannot be ascertained. This reservation is important since structural features normally exist in Phlox petals which point to the epidermis playing an important direct rôle in determining the flower form.

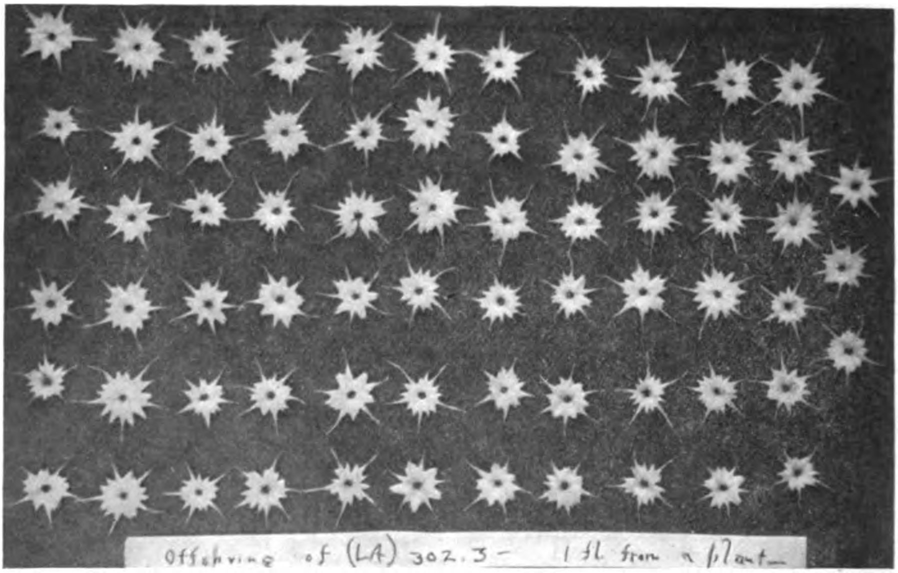


Figure 8.—An entire family descended from a *cuspidata* segregate. Each flower represents a separate plant and is typical for it.

Discussion

It is characteristic of announcements of novelties in gardening or horticultural publications that the exact circumstances surrounding the origin of a form are omitted. The account by Wittmack (1888) in the *Gartenflora* says that *cuspidata* arose from *fimbriata* by selection; as to how *fimbriata* arose nothing is written. The existence of *fimbriata* prior to *cuspidata* is to be noted, however; this points to the origin by mutation of the *cuspidata* gene in a locus of a single chromosome, presenting the new mutant character in its heterozygous expression, the *fimbriata* type; and one selection among the progeny of the original *fimbriata* should have sufficed to isolate the *cuspidata*.

C. SPRENGER (1888), who seems to have been an apothecary living near Naples, claims to have seen the spontaneous origin of *cuspidata* in his garden in April, 1888, (presumably directly, without intervening *fimbriata* stage) but the statement should be accepted with reservation. SPRENGER, by his own account, had received Phlox seeds from Quedlinburg, where *cuspidata* and *fimbriata* were first presented to the trade; it seems very possible that a stray *cuspidata* seed was what gave rise to his unexpected plant. The origin of *cuspidata* directly would most probably have required two similar mutations in distinct but homologous chromosomes.

DE VRIES (1900) lists several plants which exhibit characters completely recessive in the F_1 hybrids and reappearing in about 25 percent of the offspring of the following generation. The laciniation or fringing of the petals of a celandine (Chelidonium) proved such a character when crossed with another celandine having entire petals.

Considering plant organs homologous with petals, some data furnished by CORRENS (1912) in nettle hybrids, by LEAKE (1911) with cotton hybrids, and by GREGORY (1911) with Primula hybrids, are pertinent. CORRENS crossed a nettle with serrated leaves and one with nearly entire leaves and found serration to be a dominant character; the F_2 gave a ratio of 3 serrated to 1 non-serrated. The incised nature of the floral leaves in celandine behaves as a recessive trait in hybrids; that of the foliage leaves in the nettle is a dominant trait; while the incised floral leaves of Phlox is an intermediate. LEAKE's work concerned the genetic relationship of two types of foliage leaf, both divided but differing in the degree of division; just as in Phlox, celandine, and nettle, there was found a single factorial difference; there was intermediacy in the F_1 generation and a clear F_2 segregation on a 1:2:1 basis, as in Phlox. GREGORY's (1911) results with primroses were similar to CORRENS's with the nettles; crenate leaves proved simple dominants to the non-crenate.

Viewing the experimental results in the "Star Phloxes" from the standpoint of physiological morphology, can we conceive of the possible chemical processes involved in the formation of the fringed or incised petals of *fimbriata* and *cuspidata*? This question is really one with the whole problem of the nature of the gene and the manner by which

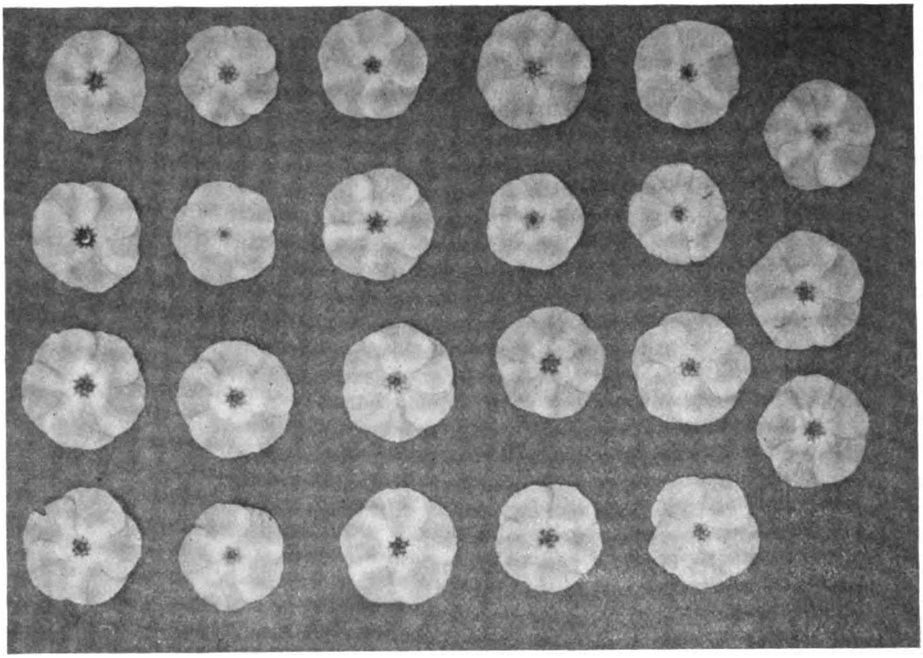


FIGURE 9.—A complete family descended from an entire-flowered segregate.

it achieves somatic expression. JENNINGS (1918), in a survey of recent genetical progress, remarks that the method of action of the genes to produce the later-appearing traits of organisms constitutes a field of research as yet little touched. Some scientists conceive the process to be enzymatic and assert that the genes are enzymes or substances which during ontogeny give rise to enzymes; that these catalyze the formation of substances distinctive chemically, which in turn are thought to form more or less directly structures or organs correspondingly distinctive. This sequence of events is a plausible one for the formation of the entire-margined flowers; it seems that in addition, in the *fimbriata* and *cuspidata* types, there is at work an inhibiting substance which retards directly the growth of the epidermal layer and to a less extent and perhaps indirectly the mesodermal layers of the petals, this retardation being less in three regions (leading to the formation of the teeth) which seem to correspond to three chief veins at the very base of the corolla lobes; the veins supply water and may be supposed to dilute the inhibitor in their vicinity, leading to tooth development. What are the reasons for assuming such an inhibitor? As pointed out before, all colored *fimbriata* and *cuspidata* flowers display a more or less irregular, narrow, colorless margin (figure 10, C); this sort of periphery never occurred in any of the entire segregates from *fimbriata* parents. Microscopic examination of free-hand sections of corolla lobes reveals at the periphery an interesting condition. All entire flowers, pure whites as well as colored, have the typical papillated epidermal cells to the very edge of the flower, as shown in B of figure 10; color where present is confined to the papillated cells, never being found in the interior. In flowers of *fimbriata* and *cuspidata* the papillated cells do not reach the edge, as shown in A of figure 10; where the papillae cease, color also ceases and hence the white or whitish border to all the incised flowers. This border would seem to be made up of mesodermal cells that had outstripped their epidermis in growth; color production in these forms is a function of the true papillated epidermis and so does not appear in these (supposedly) uncovered mesodermal cells. The conditions described suggest the presence of a growth-inhibitor in the epidermis, which restricts the size, produces the laciniation, and the white-bordered condition in the *fimbriata* and *cuspidata* flowers.

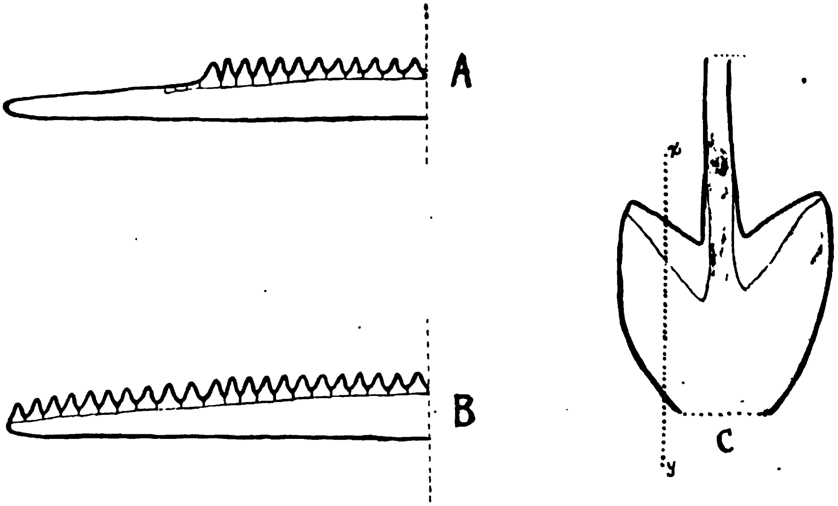


FIGURE 10.—A is diagram of the peripheral part of section of corolla lobe cut along line xy of C. This represents the condition in *fimbriata* and *cuspidata*. B represents a similar section of an entire-edged flower. C is a surface view of a portion of a *cuspidata* flower. Note the outer white edging of the flower.

SHULL (1912) has called attention to the occasional incompleteness of action of inhibitors; the fluctuational variation of several *fimbriata* flowers to entire, as already described, might be explained in this way. In our factorial summary we shall designate by I_e the factor, which in homozygous condition, causes the cuspidate form; this symbol has reference to the fact that it is apparently an inhibitor (partial) affecting the epidermal development.

The funnel-shaped Phloxes

My cultures of the funnel-shaped Phloxes were derived from seed designated commercially *"Radowitzii."* The flowers in full bloom are funnel-shaped; the first ones grown in these cultures possessed a ground

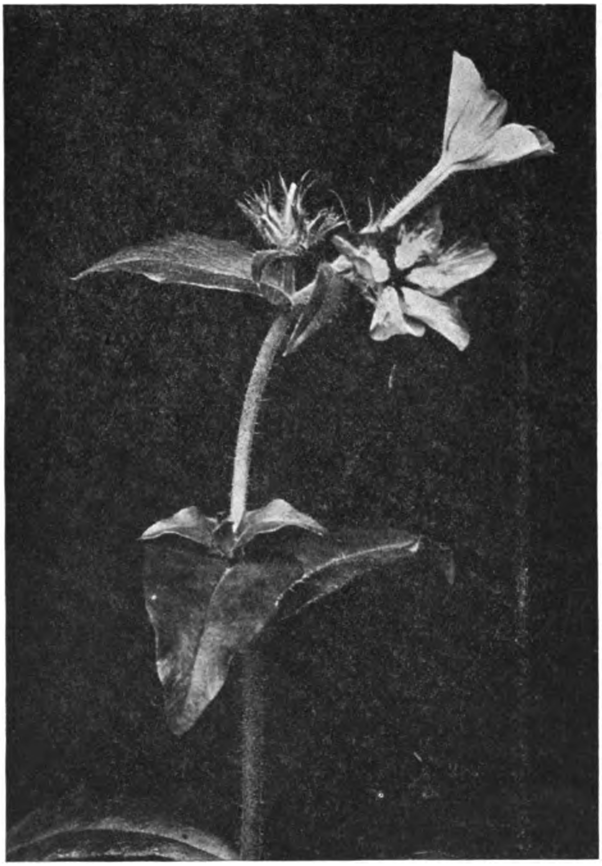


FIGURE 11.—The deep funnel type. A colored plant showing the white streaking always associated with flower color in the funnel-shaped type.

color of about a "Tyrian pink" which was streaked with white. Figure 11 shows this type of funnel-shaped Phlox; besides the characteristic shape, this type of flower commonly possesses small, irregularly distributed green streaks, and when these bits of green are at the periphery the latter becomes slightly indented. In the course of hybridization, true-breeding funnel-shaped Phloxes have arisen that are entirely white save for occasional green streakings (KELLY 1917); figure 12 is from a photograph of some of these. Other colors than "Tyrian pink" have occurred but in every case the white streaking on a colored background accompanies the funnel shape. This shape, then, as far as observations go, occurs independently of color, and always when colored is associated with white streaks on a colored ground.

My records show that selfings of 28 different funnel-shaped plants have been made at various times, giving a total of 357 progeny, and that these were unexceptionally funnel-shaped. In 1916 one family of 34 plants had its flowers only shallowly funnel-shaped; a 1917 group descended from one of these shallow-flowered plants, consisted of 20 sibs that were similarly shallow; the shallow-flowered condition is shown to the left of figure 12, while to the right is shown the commoner deep type of the writer's cultures. The hybridization work to be described was done with the latter sort (designated by an "I" in the records). All the other varieties of Phlox grown by the author had salver-shaped flowers.

The crossing between the funnel-shaped and the salver-shaped types has been made reciprocally and repeatedly. The results have always been the same; the F_1 hybrids show always a perfect dominance of the salver-shaped character. (Figure 13 shows the funnel-shaped and salver-shaped types to the left and right respectively, together with their F_1 hybrid in the centre of the picture.) Seventeen hybridizations have been made between such parents, resulting in 89 F_1 progeny, all salver-shaped. When color was involved in the F_1 hybrids the white streaking along the center of each corolla lobe proved recessive together with the funnel shape, and the irregularly distributed green streaks were likewise not apparent.

Sixteen of the F_1 plants were self-pollinated in different seasons, yielding a total of 713 offspring; this F_2 generation was composed of plants of two kinds: 534 salver-shaped and 179 funnel-shaped (see table 3). This ratio is a nearly perfect Mendelian one for a single factorial difference (with dominance).

Expectation, on this basis, would call for 534.75 salver-shaped and 178.25 funnel-shaped.

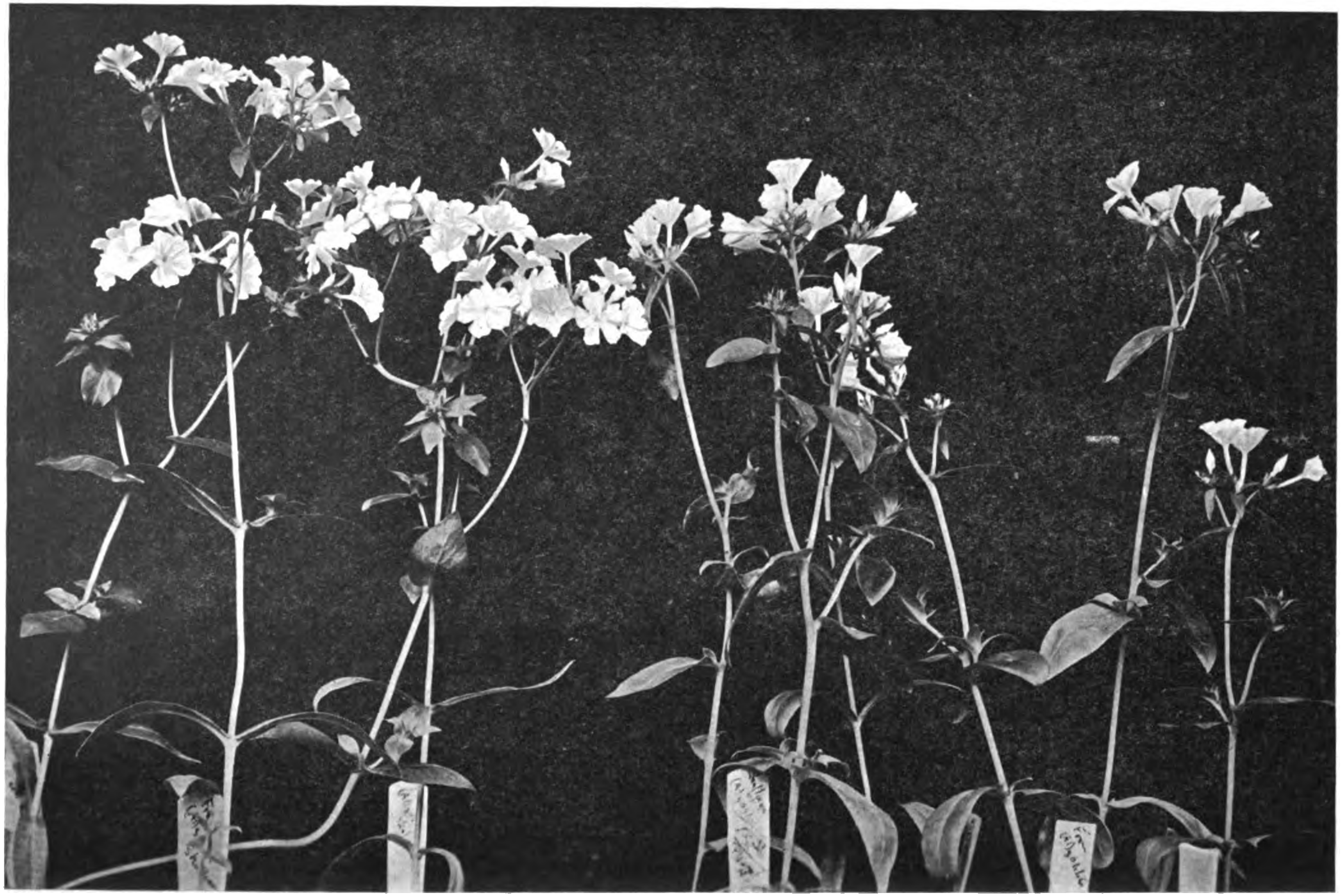


FIGURE 12.—Funnel-shaped phloxes with white flowers; shallow type to the left anddeep type to the right.

In the factorial summary we shall designate by S the factor which, either in homozygous or heterozygous condition, causes the salver-shaped flower; and by s its allelomorph which, when homozygous, leads to the funnel-shaped condition.

TABLE 3

Composition of the F$_2$ generation from crossing of the salver-shaped and funnel-shaped phloxes.

F$_1$ parent	F$_2$ progeny		Totals
	Salver	Funnel	
(AI)$_3$	21	8	29
(IA)$_2$	16	6	22
(IA)$_5$	27	5	32
(IA)$_{102}$	54	13	67
(IA)$_{105}$	60	21	81
(IA)$_{101}$	21	3	24
(IA)$_{104}$	36	8	44
(IA)$_{100}$	36	19	55
(IA)$_{106}$	36	9	45
(IA)$_{20}$	65	18	83
(AI)$_{28}$	54	20	74
(IA)$_1$	28	19	47
(AI)$_{32}$	20	6	26
(AI)$_{35}$	18	9	27
(IA)$_3$	28	11	39
(AI)$_{50}$	14	4	18
Totals	534	179	713

It has been explained already that the funnel character may be shifted about from one color type to another; it has also been evident that this character is independent of flower size, but it would seem that the larger size in funnel-shaped flowers is associated with a shallower condition. Aside from this consideration of size, however, a distinction into deeply and shallowly funnel-shaped is possible (which is genetic) as already pointed out. In the case of one plant, (IA)$_{105\cdot 1}$, the flowers, even when freshly opened, were nearly salver-shaped, but possessed the occasional green streaking and peripheral notching of petals which is so common a funnel accompaniment; this plant was classified as a funnel with the provision that its final position would rest on the nature of the progeny it produced; these turned out to be undoubted funnels; this case illustrates a decided but non-hereditary variation. Another observation was made; flowers on old plants and sometimes old flowers on young plants may become quite salver-shaped. Not all those classed as funnel-shaped, then, possessed the deep funnel shape which characterized the types used in the crossings, but there was ordinarily no trouble in separating the two groups. No attempt was made to subdivide into deeply and shallowly funnel-shaped, but all were counted together in arriving at the figures presented in table 3.

Fifteen F_3 families were grown (table 4), some of which were quite small in number as lack of time prevented the making of a sufficient number of hand-pollinations. Of ten salver-shaped F_2 parents tested, 4 gave only salver-shaped offspring and 6 produced a mixture of salver-shaped and funnel-shaped. Theory would call for 3.33 of the true-breeding and 6.66 of the heterozygous for every 10 dominants selected at random from the F_2 generation. Five funnel-flowered segregates in the F_2 generation gave only funnel-flowered progeny in the next generation, in accordance with expectation.

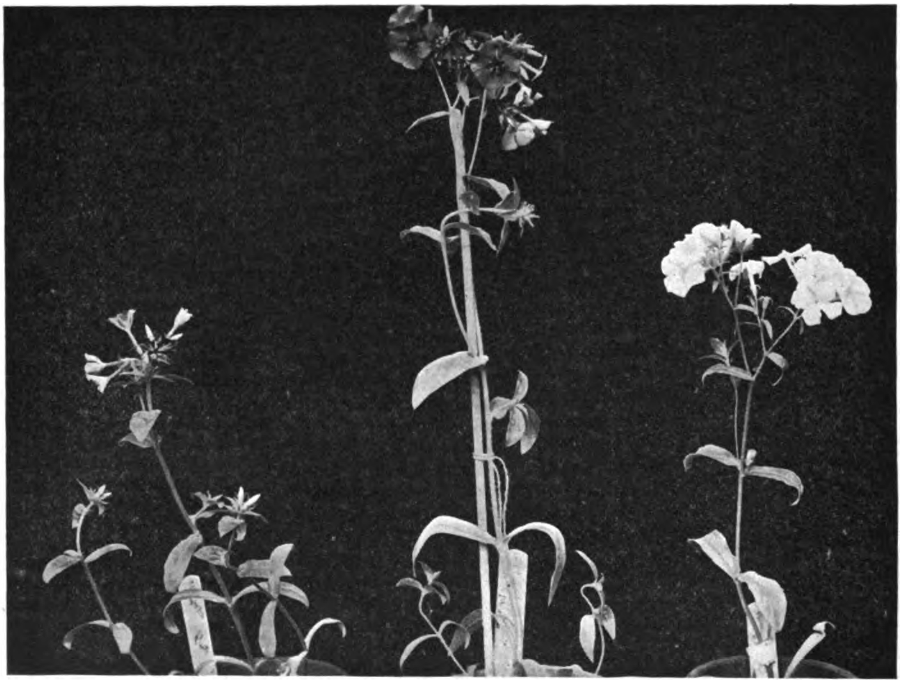


FIGURE 13.—Funnel parent type to the left; salver parent type to the right; in the center, the F_1 hybrid between them.

TABLE 4

Composition of the F_3 generation from crosses of salver and funnel phloxes.

Culture No.	Type of F_2 parent	F_3 progeny		Total
		Salver	Funnel	
$(AI)_{3.33}$	Salver	5	0	5
$(AI)_{3.3}$	Salver	6	2	8
$(IA)_{102.2}$	Salver	7	0	7
$(IA)_{100.2}$	Salver	11	2	13
$(IA)_{102.1}$	Salver	11	7	18
$(IA)_{100.3}$	Salver	7	4	11
$(IA)_{105.5}$	Salver	2	6	8
$(IA)_{102.3}$	Salver	8	2	10
$(IA)_{105.2}$	Salver	8	0	8
$(IA)_{100.5}$	Salver	15	0	15
$(AI)_{3.37}$	Funnel	0	19	19
$(AI)_{3.1}$	Funnel	0	15	15
$(IA)_{105.1}$	Funnel	0	6	6
$(IA)_{102.7}$	Funnel	0	3	3
$(IA)_{100.7}$	Funnel	0	38	38

The salver-shaped flower (with blade at right angles to the tube) represents the maximum (as far as observation goes) and also the normal grade of expansion in the species *Phlox Drummondii;* we have noted two hereditary grades that fall short of the salver condition, i.e., the shallow funnel and the deep funnel shown in figure 12. The extreme in this direction occurred in a single plant whose flowers did not open at all, the plant being perfectly cleistogamous. No seeds were obtained to test its heredity.

W. T. THISTLETON-DYER (1897) has pointed out that in the cultural evolution of *Cyclamen latifolium* a form arose whose corolla segments were spreading instead of being typically reflexed; this was a change in the grade of corolla expansion; nothing was reported as to the transmissibility of the new grade.

GENETICS OF FLOWER COLOR

Factor differences involved when a type with completely white flowers (A) was crossed with a type having dark-eyed white-bladed flowers (B)

The AB series

The parental strains have been in pedigree cultures in small numbers from season to season and inbreeding has produced no non-typical specimens. It should be remarked that not all completely white-flowered strains behave as the one used in the present crossing ($A_{51.16.8}$).

There were 19 F_1 hybrids resulting from the cross-pollination of the two parents used, six from the hybridization A × B and thirteen from the reciprocal B × A. The 19 made a decidedly uniform group, bearing flowers whose color was totally unexpected; the color of the *blade* was a full-colored type, evenly distributed, and corresponded closely with "rhodamine purple" of RIDGWAY's guide; the centre or *eye* of the flower was a more intense color. This F_1 group represents a case of complementary factors and the deep color produced resembles somewhat that of the flowers in the colored plate accompanying the original description of *Phlox Drummondii* by HOOKER (1835); the plants on which HOOKER based his account had been raised from seed imported from Texas, the wild habitat of the species. This F_1 color is much darker, however, than the color of some *Phlox Drummondii* which the author grew from seed of wild Texan plants, for which seed the author is indebted to Dr. A. B. STOUT of the N. Y. BOTANICAL GARDEN.

Six of these F_1 plants were bagged and furnished seed for an F_2 generation in 1918, consisting of 314 plants. At first glance the F_2 aggregation seemed a rather complex mixture of colors with pure white predominant, but a closer scrutiny revealed that nine types comprised the entire family. Eight of the types possessed color of some kind in the flowers and these eight very obviously fell into pairs, one of each pair being "bluer" than its mate.

Types Ia and Ib were both full- or deep-colored with the color uniformly distributed over the blade (flowers with such color distribution are sometimes called "self-colored"); neither possessed a narrow whitish or lighter-colored area immediately surrounding the eye. Ia had a blade color of rhodamine purple, just like the F_1 hybrid; Ib was the "redder" mate of Ia and had a blade color of Tyrian rose. There were 76 of type Ia and 33 of type Ib.

Types IIa and IIb also possessed a uniform distribution of their blade color (self condition), but this was much paler than in the two preceding types. The color was about "light Phlox purple" in type IIa; in its "redder" mate, IIb, the blade color was about "amaranth pink." Invariably the flowers, at least when young, possess a narrow whitish area immediately outside the eye. It is interesting to note that the IIa blade coloration is like that of the plants grown from wild seed by the writer. There were 26 IIa plants and 13 IIb plants.

Types IIIa and IIIb differ markedly from the four preceding types in the distribution of the blade color, i.e., the pigment appears to be in minute dots as though stippled on; this stippling is denser near the eye and becomes very sparse near the periphery. The pigment is also deep or full-colored. The author has fallen into the habit of calling the IIIa and IIIb plants *"duskies"* and uses this name when speaking of them in this paper. The color of the pigmented dots in the "bluer" of the "duskies" is about Rood's violet; in the "redder" of the "duskies" (IIIb) it is about Aster purple or magneta. There were 19 IIIa plants: 8 IIIb plants.

Types IVa and IVb have all color confined to an eye at the center, the blades being clear white (in the present AB series). In type IVa which resembled the "B" grandparent, the eye color is about Rood's violet, while in the "redder" member of this pair of types it is about Aster purple or magenta. There were 7 IVa plants: 2 IVb plants.

The ninth type is designated V and comprises all plants whose flowers are completely white, thus resembling the "A" grandparent. There was the unexpectedly high number of 130 plants of type V.

Colored plate 1 rather faithfully reproduces the types, together with the F_1 and parental types. Table 5 summarizes the F_2 data.

During the summer of 1919 fifty-four F_3 families were grown; many. however, were very small in numbers. A survey of the F_3 families revealed the same striking display of colors, and it was the same nine types in various proportions that made up the individual families. The color distributions are given in detail in table 6. One somewhat exceptional plant occurred in the progeny of $(AB)_{254.9}$, one of the "duskies" having the stippling dense to the very edge of the flower. The only other aberrant individual among the 889 F_3 plants occurred in the offspring of $(AB)_{251.7}$ where one of the IV group fell in the "a" subgroup when all were expected in IVb.

Certain features in the data of the F_2 and F_3 generations, as summarized in tables 5 and 6, should be emphasized:

a. The occurrence of a series of color types or classes. These have been designated by Roman numerals as already described. I is the darkest (full) self-colored type or class (by "self" is meant that the color is uniform in its distribution over the blade, exclusive of the eye region in this account). II is the paler self-colored type. III is the stippled "dusky" type or class. IV is the type or class with pigment confined to the eye at the center of the flower, the blade being clear white. V is the class with flowers completely white.

TABLE 5

Flower types in the F_2 generation of the AB series and their observed frequencies

Designation of F_2 type	Color of flower*		F_2 numbers observed
Ia	Like F_1 type; blade about rhodamine purple	Eye *not* whitish bordered (see plate 1)	76
Ib	Tyrian rose flower blade; dark eye		33
IIa	Nearly light Phlox purple; this blade resembles that of wild Phlox as grown by the writer	Eye whitish bordered (see plate 1)	26
IIb	Blade between deep rose pink and amaranth pink		13
IIIa	The "bluer" stippled "dusky"; the color stippled on blade is about Rood's violet		19
IIIb	The "redder" stippled "dusky"; the color stippled on blade is about Aster purple or magenta		8
IVa	Clear white blade; eye about Rood's violet		7
IVb	Clear white blade; eye about Aster purple or magenta		2
V	Completely white		130
		Total	314

* RIDGWAY'S "Color standards and color nomenclature" was used in color determination in this work (RIDGWAY 1912).

b. The very distinct subdivision of each color type into two subtypes marked in the tables and in the colored plate by the small letters "a" and "b". Every "a" group stands out clearly as the blue counterpart of a corresponding "b" group. This observation obviously points to a "bluing" factor capable of residing and functioning in any class of color. This independent shifting about of a gene is, of course, very characteristic of Mendelian phenomena. The absence of the "blue" factor leads to the "redder" condition.

c. The absence of forms with white eye but colored blade.

d. The theoretically very important facts that not one of the 16 F_2 plants of type II produced stippled "dusky" flowers, i.e., flowers of type

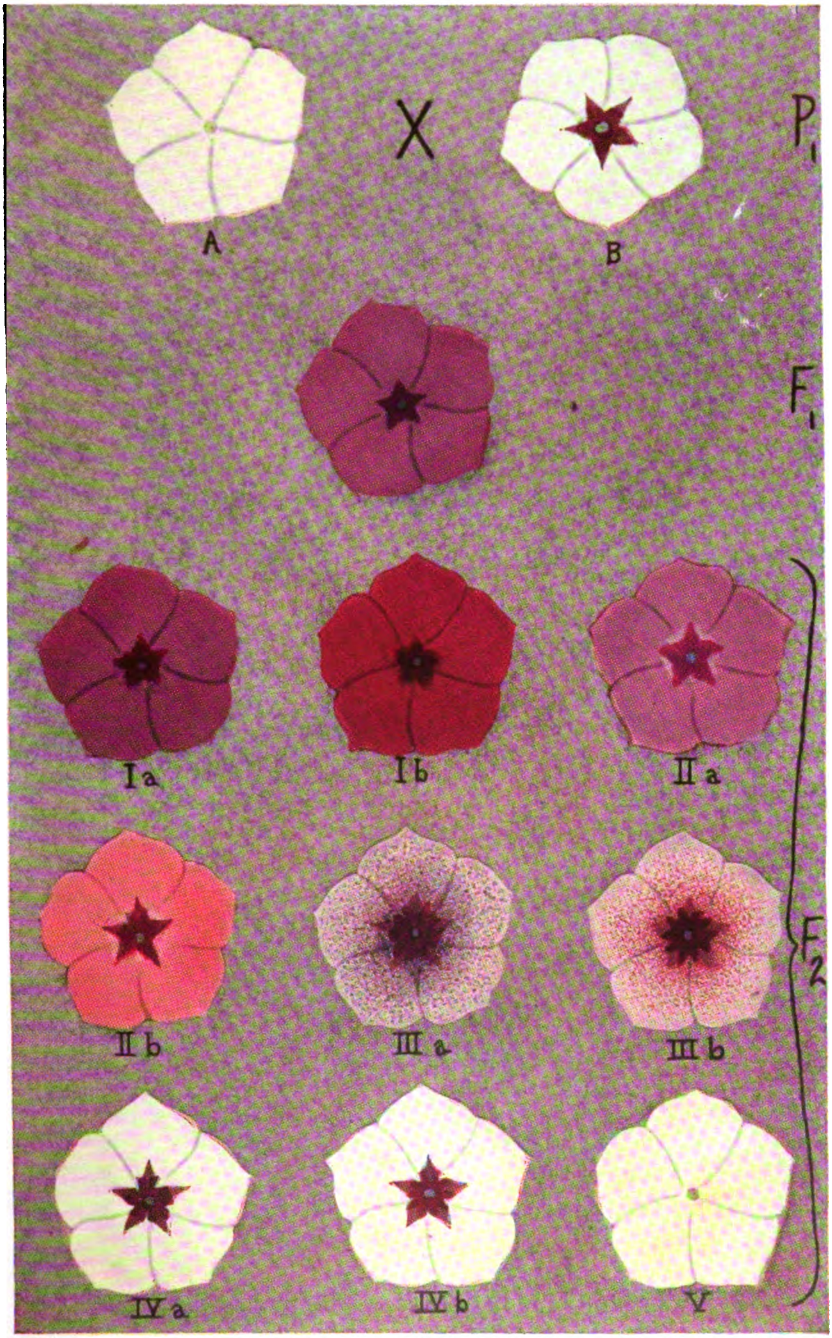
III, among its descendants; that no F_2 parent of type III on inbreeding produced any plants of type II, types II and III apparently being mutually exclusive; that type I parents are capable of producing both II and III. It would seem, then, (speaking phenotypically) that when type II meets type III it must become something else, indeed, that it must become type I. If type II were independent of type III (say epistatic to it and *not affected by its presence*) then some of the 16 F_3 families from type II parents should have contained type III plants and some should have lacked them; their total exclusion is significant. These observations receive strong support from the fuller data of the EF series, next to be described. Also to be noted is that type IV gives only IV or V on inbreeding and that type V produces nothing but type V. The following figures from the F_2 generation are of interest because of their bearing on the number of differential factors in the AB series concerned with *blade*-color formation. First, grouping type II with type I (the latter contains the differential gene for type II) we have 148 F_2 individuals; the total for all the other F_2 types (not containing the characteristic II gene) is 166; this makes a ratio of 27: 30.2. Similarly, adding type III and type I (the latter holding the differential factor for type III we obtain 136 F_2 plants; all the other F_2 plants (not containing the characteristic "dusky" gene) total 178 individuals; this gives a ratio of 27: 35.1. The numbers remind one more of a 27: 37 relationship than of a 9: 7, suggesting that a triple system of factors is prerequisite for the development of the simpler color types, i.e., types II and III.

e. The large proportion of plants with completely white flower *blades*, there being 139 in a total of 314 in the F_2 generation; that is, there were 175 with colored blades *of all types* and 139 white-bladed ones (some of these white-*bladed* plants had dark *eyes*), a ratio of 9: 7.15. Roughly, this would indicate that the F_1 hybrids were heterozygous for two factors for blade color, these factors being so interdependent that absence of either one resulted in complete absence of blade color. Closer consideration of both the F_2 and F_3 results reveals a complication, however, which imparts considerable significance to the plus deviation of 0.15 in the second term of the ratio, giving 9: 7.15 instead of 9: 7 which is expected when two complementary factors are concerned. The blade color of type II is in part dependent on a factor that has no part in forming the blade color of type III, as F_3 results indicate; and that of type III must be due in part to a specific gene non-existent in type II. These two factors *P* and *D*, characteristic of types II and III respectively, when

LEGEND FOR PLATE I

Types of flower color which appear in the AB series. The completely white-flowered and the dark-eyed white-flowered parents represented at the top (designated by A and B respectively). Immediately below these is shown the type of the F_1 hybrids. The nine lowermost flowers represent the various types which appear in the F_2 generation. [The Editor regrets that despite his persistent efforts to secure the whiteness of the flowers of the parental and lower three F_2 types, the engraver has made them distinctly yellow. The colored types are nearly correct.]

KELLY, J. P., FLOWER COLOR IN *PHLOX DRUMMONDII* PLATE 1



GENETICS 5: Mr 1920

TABLE 6

Composition of the F_3 generation of the AB series.

F_2 parent		F_3 progeny Frequencies of the several phenotypes									Totals
Designation	Type	Ia	Ib	IIa	IIb	IIIa	IIIb	IVa	IVb	V	
$(AB)_{250.26}$	Ia	5	1	2	—	3	—	—	—	4	15
$(BA)_{266.8}$	Ia	—	—	1	—	—	—	—	—	—	1
$(BA)_{266.1}$	Ia	9	—	1	—	—	—	—	—	3	13
$(BA)_{266.44}$	Ia	14	2	4	2	6	—	1	1	13	43
$(BA)_{266.2}$	Ia	6	3	—	—	—	—	—	—	5	14
$(BA)_{266.23}$	Ia	2	1	2	—	1	—	—	—	3	9
$(AB)_{250.2}$	Ia	17	2	7	1	—	—	—	—	4	31
$(AB)_{251.12}$	Ia	1	—	2	—	—	—	—	—	—	3
$(AB)_{251.2}$	Ia	6	3	3	—	—	—	—	—	5	17
$(BA)_{266.35}$	Ia	16	—	3	—	2	2	1	1	10	35
$(BA)_{266.22}$	Ia	12	3	6	2	—	—	—	—	6	29
$(AB)_{254.16}$	Ib	—	2	—	—	—	—	—	—	—	2
$(AB)_{250.8}$	Ib	—	3	—	—	—	—	—	—	3	6
$(AB)_{250.1}$	Ib	—	3	—	1	—	1	—	—	4	9
$(BA)_{261.9}$	Ib	—	14	—	—	—	7	—	—	9	30
$(BA)_{266.25}$	Ib	—	1	—	—	—	—	—	1	—	2
$(BA)_{266.15}$	Ib	—	5	—	—	—	—	—	—	1	6
$(BA)_{266.46}$	Ib	—	—	—	—	—	3	—	—	2	5
$(BA)_{266.7}$	Ib	—	10	—	—	—	—	—	—	—	10
$(BA)_{261.15}$	Ib	—	6	—	—	—	5	—	—	4	15
$(AB)_{250.18}$	Ib	—	2	—	—	—	—	—	—	2	4
$(BA)_{266.43}$	Ib	—	15	—	—	—	5	—	—	18	38
$(BA)_{266.16}$	Ib	—	15	—	—	—	4	—	—	12	31
$(BA)_{266.12}$	IIa	—	—	10	—	—	—	—	—	3	13
$(BA)_{266.18}$	IIa	—	—	4	—	—	—	—	—	3	7
$(BA)_{266.31}$	IIa	—	—	3	—	—	—	—	—	4	7
$(AB)_{250.5}$	IIa	—	—	3	—	—	—	—	—	2	5
$(BA)_{269.6}$	IIa	—	—	1	—	—	—	1	—	3	5
$(BA)_{266.21}$	IIa	—	—	1	—	—	—	2	—	6	9
$(AB)_{251.5}$	IIa	—	—	7	9	—	—	—	—	13	29
$(BA)_{266.20}$	IIa	—	—	17	8	—	—	—	—	8	33
$(BA)_{261.3}$	IIa	—	—	17	2	—	—	2	—	14	35
$(AB)_{251.6}$	IIb	—	—	—	18	—	—	—	8	20	46
$(BA)_{269.12}$	IIb	—	—	—	21	—	—	—	—	—	21
$(AB)_{250.30}$	IIb	—	—	—	9	—	—	—	—	5	14
$(AB)_{251.39}$	IIb	—	—	—	5	—	—	—	1	2	8
$(AB)_{251.7}$	IIb	—	—	—	8	—	—	—	2	8	18
$(AB)_{250.20}$	IIb	—	—	—	3	—	—	—	2	—	5
$(AB)_{269.11}$	IIb	—	—	—	6	—	—	1	2	9	18

TABLE 6 (continued)

F_2 parent		F_3 progeny Frequencies of the several phenotypes									Totals
Designation	Type	Ia	Ib	IIa	IIb	IIIa	IIIb	IVa	IVb	V	
$(BA)_{261.2}$	IIIa	—	—	—	—	21	—	4	—	—	25
$(AB)_{254.9}$	IIIa	—	—	—	—	3	—	—	—	3	6
$(AB)_{251.16}$	IIIa	—	—	—	—	20	—	5	—	—	25
$(BA)_{266.11}$	IIIa	—	—	—	—	10	—	—	—	3	13
$(BA)_{260.5}$	IIIb	—	—	—	—	—	7	—	4	11	22
$(AB)_{254.8}$	IIIb	—	—	—	—	—	—	—	1	4	5
$(BA)_{266.9}$	IIIb	—	—	—	—	—	5	—	—	4	9
$(AB)_{250.8}$	IIIb	—	—	—	—	—	7	—	1	4	12
$(BA)_{266.10}$	IIIb	—	—	—	—	—	11	—	—	10	21
$(AB)_{251.20}$	IVa	—	—	—	—	—	—	13	—	11	24
$(BA)_{266.4}$	IVa	—	—	—	—	—	—	7	—	5	12
$(BA)_{266.8}$	IVb	—	—	—	—	—	—	—	12	5	17
$(BA)_{260.36}$	V	—	—	—	—	—	—	—	—	10	10
$(BA)_{266.6}$	V	—	—	—	—	—	—	—	—	16	16
$(BA)_{266.5}$	V	—	—	—	—	—	—	—	—	31	31
										Total	889

present together give type I. All blade color, in other words, is here due to the presence (among other things) of either one or both of these two factors specific for II and III, and either or both of these factors will be present in 15/16ths of the F_2 zygotes; for rough estimation both may be counted as single homozygous color determiners leaving the number of white-bladed plants to be determined very largely by two other independent genes, *E* and *A*, which are ineffectual unless both are present. In an exact consideration, of course, it is to be noted that 1/16th of the zygotes will lack the two genes differential for types II and III and this should add slightly to the group of white-bladed individuals, as observation actually showed. Blade color of the simplest sort, then, (that of types II or III) is dependent on three differential factors in the AB series, although the F_2 figures roughly support a bifactorial difference for the relationship of blade color (all sorts collectively) to lack of blade color.

With the above features of the experiments in mind we find it easy to frame a provisional hypothesis by which to interpret the data. We may assume that three distinct factors were essential in the AB series for the appearance of either of the two simple sorts of blade color (types II and III), the absence of even one being sufficient to produce colorlessness in the blade. The most current theory concerning the formation of anthocyanin pigments is based on the work of PALLADIN and of WHELDALE (1916) and applying their conclusions to our factors we may suppose one to be a chromogen-producing factor, *P* (or *D*); the second factor, *E*, may be thought to give rise to an enzyme, possibly of the nature of an oxydase which acts on the chromogen to form anthocayanin color, provided there is present, in turn, a third factor which we may call *A* and whose function it is to produce an activator for the enzyme; this activator when fully formed might be supposed to have the constitution of an organic peroxide. When all three are present the color produced would be that of type IIb if the chromogen factor present happened to be *P*; if the chromogen factor were *D* (described below), then the color formed would be the stippled "dusky" type IIIb.

Further, we must obviously assume a factor *B* whose presence accounts for the "bluer" member of each pair of subtypes. For example in type II, the presence of this factor *B* would give type IIa, its absence, type IIb. This factor also affects the eye color, as stands out with especial clearness in type IV where no blade color is present.

A factor *D* that produces the "dusky"-appearing blade color, magenta or Aster purple, is assumed; it is conceived to give rise to an added blade chromogen; it is also conceived to be linked very closely with a factor that causes a stippied distribution of the "dusky" color. A further interesting characteristic imputed to factor *D* is that it and the factors that produce type II are complementary and give type I; speaking phenotypically we mean to say that II + III = I. More exactly expressed: *D* and *P* in the presence of *E* and *A* give the type I coloration.

Flowers of Phlox have not been chemically examined to test the assumption that multiple chromogens are present; that it is not unreasonable is attested by WHELDALE's account (1916, page 69) of the pigments in the snapdragon; she found that the crimson and bronze varieties each contained two chromogens (flavones), i.e., apigenin and luteolin.

In Phloxes that bear completely colored flowers the pigment in the outer part of the expanded corolla (the "blade") is distinguishable from that of the central part immediately surrounding the throat (the "eye"). Variation in the species, too, has produced forms with colored blades and colorless eyes; and *vice versa*. Furthermore, proper crosses show the blade color to be segregable from the eye color. It is plausible to suppose that the chromogen bases in the two regions are different and produced by distinct factors. The genes *P* and *D*, before described, are conceived to be concerned in chromogen production in the outer or blade part of the flower and the factor *M* is postulated for chromogen production in the eye. Since no colored-bladed white-eyed flowers occurred in this AB series the *M* must have been homozygous in these hybrids and contributed by both of the original parents. The factor *M*, in other words, is not differential in the AB series and its somatic expression as the flower eye is not particularly taken into account here; the conditions determining blade color constitute the special interest in this series.

By way of summary, the pertinent factors contained in the two plants originally crossed are listed herewith:

PARENT $A_{51.16.8}$	PARENT $B_{212.5}$
P, chromogen factor for the IIb type of blade color.	*p*, the absence or allelomorph of *P*.
e, absence or allelomorph of *E*.	*E*, factor for an enzyme; in presence of factors for a chromogen and activator, *E* will produce anthocyanin.
a, absence or allelomorph of *A*.	*A*, factor for an activator of *E*.
D, chromogen factor for the IIIb type of color. In the AB and EF series this color is stippled on.	*d*, absence or allelomorph of *D*.
b, absence or allelomorph of *B*. The presence of *b* in homozygous condition gives the "redder" colorations.	*B*, "bluing" factor.

M, a factor that produces the dark eye is thought to be present in both parents; if it be a chromogen-producing factor it will need the presence of both *E* and *A* for expression.

As already explained, when *P* and *D* are present together (with *E* and *A*) they interact and give a composite effect (type I).

Five independent pairs of factors are postulated in this scheme. Table 7 lists their possible combinations with the expected numbers for

TABLE 7

The possible factor combinations for the F_2 generation of the AB series.

Factor combinations					Phenotypic formulae	Phenotype	Theoretic numbers
P	*E*	*A*	*D*	*B*	*PEADB*	Ia	243
				b	*PEADb*	Ib	81
			d	*B*	*PEAdB*	IIa	81
				b	*PEAdb*	IIb	27
		a	*D*	*B*	*PEaDB*	V	81
				b	*PEaDb*	V	27
			d	*B*	*PEadB*	V	27
				b	*PEadb*	V	9
	e	*A*	*D*	*B*	*PeADB*	V	81
				b	*PeADb*	V	27
			d	*B*	*PeAdB*	V	27
				b	*PeAdb*	V	9
		a	*D*	*B*	*PeaDB*	V	27
				b	*PeaDb*	V	9
			d	*B*	*PeadB*	V	9
				b	*Peadb*	V	3
p	*E*	*A*	*D*	*B*	*pEADB*	IIIa	81
				b	*pEADb*	IIIb	27
			d	*B*	*pEAdB*	IVa	27
				b	*pEAdb*	IVb	9
		a	*D*	*B*	*pEaDB*	V	27
				b	*pEaDb*	V	9
			d	*B*	*pEadB*	V	9
				b	*pEadb*	V	3
	e	*A*	*D*	*B*	*peADB*	V	27
				b	*peADb*	V	9
			d	*B*	*peAdB*	V	9
				b	*peAdb*	V	3
		a	*D*	*B*	*peaDB*	V	9
				b	*peaDb*	V	3
			d	*B*	*peadB*	V	3
				b	*peadb*	V	1
							1024

each combination in every 1024 individuals of the F_2 generation; the phenotype to which each combination belongs is also indicated. In every 1024 individuals then, theory would call for 243 Ia:81 Ib:81 IIa:27 IIb:81 IIIa:27 IIIb:27 IVa:9 IVb:448 V.

Table 8 gives the theoretical expectations based on a total of 314 plants alongside of the observed results. Hypothesis leads us to expect the ratio of the more "bluish" to the more "reddish," i.e., of "a" types to "b" types, to be 3:1. In the F_2 generation, however, actual experiments gave

76 Ia : 33 Ib;
26 IIa : 13 IIb;
19 IIIa : 8 IIIb;
7 IVa : 2 IVb.

The ratio of any "a" type to any "b" type averages about 2.3 : 1. There may be some selective elimination affecting the "blue" zygotes; or, the theory of linkage may be invoked to explain this ratio, leading to the adoption of a modified view of the bluing factor. By assuming that instead of a single factor, *B*, conditioning the blue color of the classes, there are two linked genes B_1 and B_2, the presence of both of which simultaneously is necessary to give the bluish tint; and by supposing that the linkage value is 90 percent, i.e., that one crossover gamete is formed to every nine non-crossover, there would be then a ratio of "blue" to "red" of 2.3 : 1. This bifactorial interpretation for the formation of the bluish series has not been experimentally tested, either in the AB series or in the EF series next to be presented; if it be the true explanation crossings of the "red" segregates should recombine B_1 and B_2 in some of the hybrids and produce "blues," a result not to be expected on the single-factor hypothesis for bluing. The theoretical numbers in table 8 were based on a single dominant factor for bluing; these calculated numbers and the numbers observed in the experiments are repeated in table 14 (left half), together with the amended theoretical ratios, these last being based on the *pair of linked factors* for bluing just discussed. The correspondence of observation and calculation is very good. For the nine types observation furnished this ratio,—

76 : 33 : 26 : 13 : 19 : 8 : 7 : 2 : 130

while theory for the corresponding types gives about,—

69 : 29 : 23 : 9 : 23 : 9 : 7 : 3 : 137. (See table 14 for *exact* figures.)

For these ratios χ^2 is about 4.3; n' is 9; and by ELDERTON's table, P is .82.

In postulating a set of five independent genes to account for the genetic behavior described, the question of the number of chromosomes becomes of interest. Sections of the root-tips of seedlings reveals that there are fourteen chromosomes in the somatic cells of *Phlox Drummondii.*

In connection with the three genes considered prerequisite to any color formation, an attempt was made to describe them in terms of the chemical activities supposed to be associated with them; and the more commonly accepted of the views for the formation of anthocyanin were embodied in that factorial description. Certain investigators, however, notably COMBES, EVEREST, and WILLSTÄTTER, start from the same chromogen and arrive at anthocyanin by a different means, i.e., by a reducing

TABLE 8

Actual and theoretical composition of the F_2 generation in the AB series.

F_2 phenotype	Observation	Calculation for 314 plants
Ia.........	76	74.5135
Ib.........	33	24.8378
IIa.........	26	24.8378
IIb.........	13	8.2792
IIIa.........	19	24.8378
IIIb.........	8	8.2792
IVa.........	7	8.2792
IVb.........	2	2.7597
V	130	137.3747
Totals	314	313.9984

For ratios in this table χ^2 is 7.6; n' is 9; P is .47.

process. In the case of *Phlox Drummondii* the author has found that the water extract of white flowers, when treated with nascent hydrogen, becomes pinkish, that is, of a color in quality like that of the anthocyanin in the living flower; oxidative agencies did not achieve this result. No chemical analysis of the artificial pink pigment was made. Some reducing system may perhaps more truly represent the mechanism of color formation. It is known, for example, that methylene blue in milk becomes reduced when a little acetaldehyde is added; this is because of the presence of a reducing enzyme in the milk. To parallel this in the Phloxes we could assume a chromogen that is reduced by a reducing enzyme to an anthocyanin in the presence of some activator like an aldehyde. The substitution of this system for the one already set forth will not alter the nature of the explanation given.

An examination of table 6 shows that among the F_3 progeny of various Ib parents there is an unexpected dearth of plants falling in type II; an aberrant number of stippled "duskies" (type III) compared to dark-eyed whites (type IV), however, is just what would be expected on the scheme proposed. It would seem that in these cases, chiefly parents homozygous for stippled "dusky" were inadvertently chosen and the presence of factor *D* in all gametes would preclude a type II or type IV plant from appearing.

Do the experimental facts of the F_3 generation as summarized in table 6 bear out the hypothesis that three factors must be present before the production of either of the simpler sorts (II or III) of anthocyanin in the blade takes place? Before considering the details of the table in an attempt to answer the question, it should be recalled that there are *two triple systems* in this AB series for production of simple blade color. These are distinguished supposedly by the chromogen factor in each. The two systems are

$$P + E + A, \text{ and}$$
$$D + E + A.$$

The F_2 "duskies" (type III) should give origin to F_3 families of three kinds, if a triple-factor hypothesis be true, depending on whether the F_2 parent was heterozygous for one, two, or three of the three requisite factors. With heterozygosity in just one gene there should be about one in four that is non-dusky; with heterozygosity in two of the genes there should be seven out of every sixteen offspring that are non-dusky; where an F_2 parent is heterozygous in all three genes there should be 37 non-dusky in every 64 offspring. Similar considerations hold in the case of F_2 individuals of type II. Type I, as has been pointed out, is a composite of II and III. The F_3 test of the hypothesis of triple systems of complementary genes for the production of the two simple types of blade color is based on a regrouping of the F_3 data: first, F_2 plants of types II and III are considered and their progeny are classified into those containing simultaneously P (or D) $+ E + A$, and those not. It should be remembered that a stippled "dusky" (III) or a dark-eyed white (IV) lacks the factor P for blade color; plants with completely white flowers lack factor E or A or both. When dealing with the system $P + E + A$, therefore, we must count stippled "duskies" and dark-eyed whites along with complete whites as being all without the whole set of factors for type II coloration, although another blade color may be present. The data on the groups from F_2 plants of types III and I bear on the second of the triple systems and the F_3 progeny are grouped into "dusky" or "dusky"-containing and non-"dusky." Table 9 gives the percentage of plants without $P + E + A$ simultaneously, or without $D + E + A$ simultaneously. The families with less than 15 members are omitted because of the uncertainty connected with such small numbers.

The data of table 9 are thrown together in a frequency polygon in figure 14. One F_2 individual, $(BA)_{269.1}$, must have been homozygous for P, E and A, since no whites appeared among its F_3 descendants; this individual causes the small peak at 0 percent. The remainder of the curve shows three maxima; the first somewhere about 25 percent, the second near to 43.75 percent, and the third near 57.81 percent. This is in accordance with expectation on a three-factor hypothesis; for some

TABLE 9

Data of the F_3 generation of the AB series regrouped to show bearing on the hypothesis of multiple factors for the production of coloration of types II and III.

F_2 parent	F_3 progeny	
	Percentage without $P+E+A$	Percentage without $D+E+A$
$(AB)_{250.25}$	46.6	40.0
$(BA)_{266.44}$	48.8	48.8
$(AB)_{250.2}$	12.9	38.7
$(AB)_{251.2}$	29.4	47.0
$(BA)_{266.35}$	45.7	42.8
$(BA)_{266.23}$	20.6	48.2
$(BA)_{261.9}$	53.3	30.0
$(BA)_{261.15}$	60.0	26.6
$(BA)_{266.43}$	60.5	47.3
$(BA)_{266.16}$	51.6	38.7
$(AB)_{251.5}$	44.8	
$(BA)_{266.20}$	24.2	
$(BA)_{261.8}$	45.7	
$(AB)_{251.6}$	60.8	
$(BA)_{269.12}$	0.0	
$(AB)_{250.20}$	55.5	
$(AB)_{251.7}$	66.6	
$(BA)_{261.2}$		16.0
$(AB)_{251.16}$		20.0
$(BA)_{269.5}$		68.1
$(BA)_{266.10}$		47.6

F_2 colored individuals should be homozygous for all three essential factors and be incapable of giving white descendants; some should be heterozygous in one of the factors and homozygous in the other two and behave as simple monohybrids, giving rise to 5 percent of white progeny; some should be heterozygous in two factors and homozygous in one, producing an F_2 ratio of 9 colored to 7 whites, in other words, give 43.75 percent of whites; the remainder should be heterozygous in all three of the necessary genes and give a ratio of 27 colored to 37 white, which amounts to 57.81 percent of white offspring.

Our calculations lead us to expect in the F_2 generation 27 plants containing genes for a particular blade color in every 64, on a three factor basis for this blade color; and of these 27 pigmented plants, 1 should be homozygous for all three genes, 6 should be homozygous for 2, 12 should be homozygous for one factor, and 8 should be heterozygous

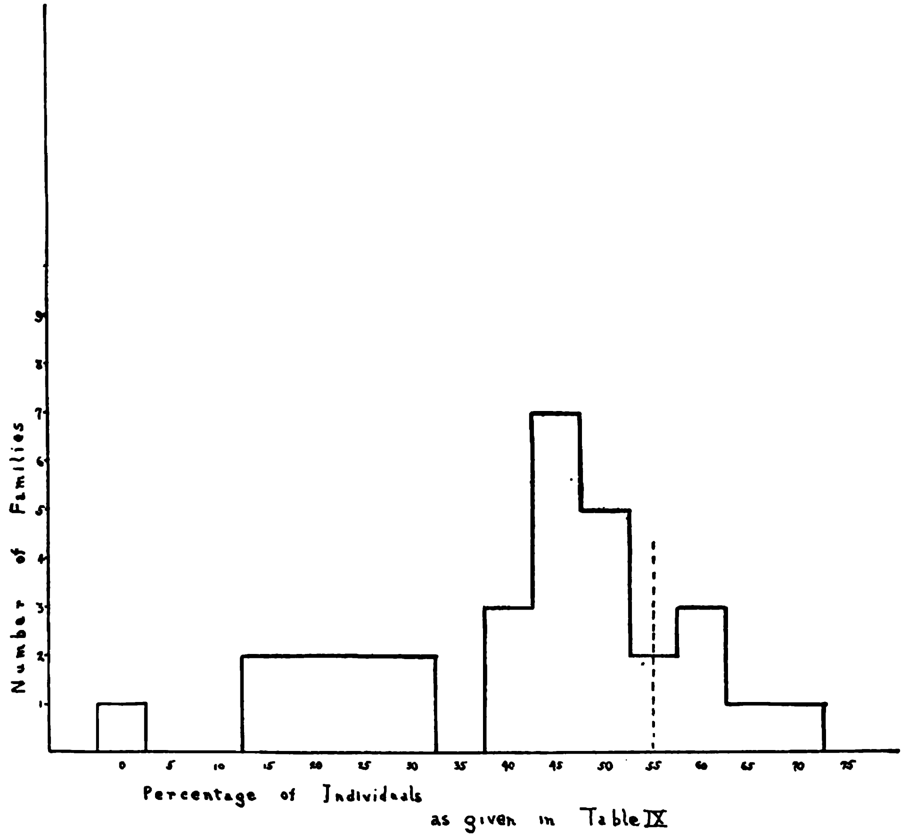


FIGURE 14.—Abscissal numbers show proportion of each F_2 family not containing simultaneously $P+E+A$ or $D+E+$A. Ordinates give number of families for various abscissal percentages.

for all three. Table 9 and figure 14 give experimental data on the progeny of 31 F_2 plants, and for this number theory would call for 1.1 : 6.8 : 13.7 : 9.1 plants of the respective genotypic constitutions just enumerated. Our observations gave for these types respectively 1 : 8 : 16 : 6, which is a fairly good correspondence with expectation. (The determination of 16 and 6 for the last two classes was obtained by arbitrarily separating the bimodal polygon constituting the right of figure 14 at the 55 percent point.)

Discussion of compound characters

A brief consideration of "compound characters" (BATESON 1913, p. 60), both where the component elements are capable of independent expression and where they become visible only when present and interacting, seems desirable at this point. Contributions of EAST (1912), EAST and HAYES (1911), and EMERSON (1918) have demonstrated that the simultaneous presence of as many as four distinct factors is prerequisite to the formation of aleurone color in maize. These give rise to a reddish pigmentation. The presence of a fifth (dominant) factor causes the production of a bluish coloration.

GREGORY'S (1911) work on the Chinese primrose (*Primula sinensis*) reveals an interesting similarity to the AB series just described in the Phloxes; he found that crossing full-colored kinds to a white gave an F_2 composed of four types: a, Full-colored selfs; b, paler-colored selfs; c, deep stippled types which he calls "Sirdars"; and d, complete whites. He did not get beyond the F_2 generation and left undecided the place of the paler selfs in the genetic scheme for the primrose; that is, he could not say whether the paler selfs were an independent series of colors in relation to full-colored selfs or whether they resulted "from the resolution of the combination of factors to which the full color is due." As supporting the latter alternative he pointed out that all the full-colored selfs that were crossed with a white variety called "Snowdrift" gave the paler self-colored types in the F_2 generation; and that two heterozygous "Sirdars" threw only "Sirdars" and whites. Concerning the "Sirdars," GREGORY considered these to differ from full-colored selfs only by a gene which made for uniform or non-stippled color distribution. There are distinct bluish and reddish subtypes of primroses but unlike the condition in the Phloxes, the "blue" is recessive to "red" and this is the situation also in *Lychnis dioica* (SHULL 1912). Data collected through the F_3 generation demonstrated the clear interaction-effects between the eye of the flower of primrose and the length of the style; a large diffuse eye acted as an inhibitor to the development of the long style.

The snapdragon (*Antirrhinum majus*) has been referred to as the most analyzed of plants. BAUR (1914) points out the necessity of two factors, B and F, for the production of anthocyanin of any kind. In a list of the more important factors of this plant he mentions three, M, M_1 and L, that intensify the color produced by B and F; he says (page 105) that a plant of the genetic formula $BBFFmmM_1M_1LL$ and one of the formula $BBFFMMm_1m_1ll$ are both of about the same color, i.e., a dark flesh color. The combination of the two with formula $BBFFMMM_1M_1LL$ is a plant that is deep velvety blackish-red ("satt sammetig schwarzrot"). This is analogous to what appears to be the case in *Phlox Drummondii* where the combination in one plant of the factors producing the stippled "dusky" and the paler-colored selfs gives the full-colored selfs. The snapdragons have also a dominant bluing factor as in the phloxes.

In the sweet pea (*Lathyrus odoratus*) it has been long known that two factors, C and R, must be simultaneously present before any sort of anthocyanin can be formed. In addition, genetic grades of color have been described by BATESON and PUNNETT (BATESON 1913). There is the purple picotee or tinged type which differs from the deep purples by a single factor necessary for the full color of the latter. The corresponding pink picotee or tinged differs because of the same factor from the deep pinks. The condition here is not like that in the phloxes where the gene that effects an intensification of the paler self-colored types is capable of visible expression in the absence of the paler colors. The gene in sweet peas responsible for the "bluer" flower colors is a dominant one, as it is in the phloxes.

Stocks(Matthiola species) have been investigated especially by SAUNDERS (BATESON and SAUNDERS 1902; BATESON, SAUNDERS and PUNNETT 1905, 1906, 1908). Here also two factors must be present together for any anthocyanin development; a dominant bluing factor, too, is present giving rise to a "blue" and a "red" series of colors; certain light kinds, flesh-colored and lilac-colored, have appeared which are not well known from the standpoint of their relationships to each other and to the other colors. An interesting example of a compound character due to the interaction of several known genes occurs in stocks. The hairy condition is attributed to the factor *H* for hairs but this never achieves somatic expression unless the factors *C* (for cream chromogen) and *R* (for red coloration) are simultaneously present.

Among animals a classical case of a compound character and of spectacular factor interactions is that of the walnut comb in fowls (BATESON, SAUNDERS and PUNNETT 1905, 1906; also BATESON 1913). Just as in the phloxes where the resolved components of the full-colored selfs exhibited themselves independently as stippled "dusky" and paler self-colored types, so here the components of walnut comb, by themselves, attain independent expression in the form of the rose and the pea combs.

It seems needless to cite further cases. The whole trend of recent genetics is to get away from the idea of a gene independently and exclusively conditioning some "unit character" in the soma; and it is likely that factor interaction in a less spectacular manner than in the cases just discussed must be very common in the living world.

THE CROSSING OF BRILLIANT "RED" OR CARMINE (E) BY PURE CREAM (F) —THE EF SERIES

The red parent (cultural designation $E_{47.1.1}$) and the cream parent ($F_{51.2.8}$) were both members of lines that had been the two preceding seasons in pedigree culture and known to be true-breeding for red and cream respectively. According to RIDGWAY'S "Color standards and nomenclature" the cream of the blade was marguerite yellow while the red was difficult to match in that guide but it seemed to be about a brilliant pomegranate purple or carmine.

The F_1 generation consisted of fifteen plants that made a remarkably uniform group; the outer blade color of the flowers was very close to RIDGWAY'S rhodamine purple while the eye region was deeper. The F_1 color seemed not so intense as that of the carmine (E) parent and it was decidedly "bluish" or purplish, indicating that the cream parent (F) had introduced both a diluting and a bluing factor.

Several of the F_1 individuals were selfed and gave seed for an F_2 generation of 530 plants, grown in 1918. The group as a whole presented a striking variety of colors ranging from some that were deeper than the F_1 type to those completely white. Closer scrutiny revealed *a remarkably close parallelism to the conditions already described in the AB series.* A few plants looking just like the carmine parent occurred, and also paler reddish self-colored ones, more reddish stippled duskies, reddish dark-eyed kinds (differing from their AB analogues by usually possessing a characteristic very light stippling external to the eye). For each of the types in this reddish series a closely corresponding type in a bluish series was present. Again, a large number have flowers entirely without anthocyanin. The older flowers of the "duskies," of those with color confined to the eye, and the completely non-anthocyanin forms possessed visibly either cream or white plastids; which of the other types were underlaid with cream and which with white could not be determined, as a rule, by inspection; the cream was so light that the quality of the colors of the selfs was not appreciably affected, if at all.

The visibility of cream in the lighter types was one difference setting off the EF series from the AB series. Another has also been referred to, namely, the appearance of dark-eyed forms with blades lightly stippled and which were quite distinct from the relatively heavily stippled "duskies"; the light stipples graded over into the dark-eyed forms with perfectly clear blades and the separation of the two was made with considerable difficulty and much doubt. There was also perceptible within each main group quite minor sub-groups. When recording the F_2 generation the two groups of full-colored selfs whose eyes were not whitish- or lightish-bordered (Ia and Ib) and the groups of the paler-colored selfs whose eyes were always whitish- or lightish-bordered (IIa and IIb) were each subdivided into a darker and lighter subdivision; the F_3 observations made in 1919 indicate that a subdivision of each of the afore-mentioned groups into a darker, a medium, and a lighter would have been more in accordance with the facts; the darker of these three subdivisions (as it occurs in all of the main groups Ia, Ib, IIa, IIb) is not only darker but also "redder" than the medium and lighter ones, showing that the factor concerned here, D_6 is a slightly reddening as well as a slightly intensifying gene; the "lighter" subdivision is conceived as due to the minor diluting factor, D_4, while the medium arises when there is neither D_6 nor D_4 present. Lastly, this distinction should be emphasized, that, while the main groups among the EF and AB series are similar, in the EF series they are more intense in color. It is thought that the factors determining the color types and their relative distinctions are identical in the two groups but that they play upon a genotypic residuum in the EF series that is different from what it is in the AB series. Expressed in other words, the modifications noted in the EF series are probably due to the occurrence of one or more additional factors which have not yet been investigated.

A summary of the F_2 observations in the EF series is presented in table 10. No separate colored plate was furnished for the EF series since the F_2 types that arose so closely paralleled those of the AB series pictured in plate 1, and the same designations are used in the EF and AB series for corresponding types. This statement applies also to the type designations in the tables in these two series.

During the summer of 1919 eighty-one F_3 families were grown comprising a total of 3333 individuals. The EF plants were more readily self-pollinated than the AB plants, and the families therefore run larger. During 1919 the greater size of the cultures and a limitation of time prevented the subsequent follow-up examination to include late-blooming members of the families but *complete* observations on the F_2 generation the preceding year showed that late blooming is not associated with any particular type of flower; that late-bloomers represent a random sample of the population so far as the characters under investigation are concerned.

TABLE 10

Flower types in the F_2 generation of theEF series and their observed frequencies.

Designation of F_2 type		Color of flowers		Observed F_2 numbers	
Ia	lighter	Like F_1 color and those close to it; blade close to rhodamine purple	No lighter area near border of eye	103	131
	darker	Deeper than F_1; blade about a brilliant Aster purple		28	
Ib	lighter	Blade about tyrian rose		57	62
	darker	Blade about bright carmine or bright pomegranate purple (= E parent type)		5	
IIa	lighter	Blade from rhodamine purple to true purple	Eye bordered by whitish or lighter area	59	73
	darker	Blade perhaps between rhodamine purple and Aster purple		*14	
IIb	lighter	Blade between tyrian pink and tyrian rose		24	28
	darker	Blade very close to tyrian rose		4	
IIIa		Stippled "dusky"; color about pansy violet or Rood's violet			43
IIIb		Stippled "dusky"; color about Aster purple or magenta			27
IVa	stipple	Violet-eyed with relatively few stipples or dots on blade		14	28
	clear	Same as last but with apparently undotted or unstippled blade		14	
IVb	stipple	Aster purple or magenta eye and blade with sparse stippling		3	14
	clear	Same as last but with apparently undotted or unstippled blade		11	
V	white	Blade and eye completely white		90	124
	cream	Blade and eye completely cream		34	
				Total	530

* Four allotted to this group whose classification was somewhat doubtful.

A complete summary of the data of the F_3 families (together with an indication of the types of the F_2 parents) is given in table 11. The following features of the data of the F_2 and F_3 generations as summarized in tables 10 and 11 should be emphasized:

a. A smaller proportion of plants in this series than in the AB series whose flower blades lack anthocyanin, these having blades white or cream, or at most merely lightly stippled with anthocyanin. There were 166 such in 530, or a ratio of 1 : 2.19.

b. The occurrence again of a series of types of coloration just as was found in the AB series and in their larger features analogous to those found in that series.

c. The clear division again of each main group into two, a relatively "bluer" denoted by the letter "a" and a relatively "redder" denoted by "b". Certain finer sub-divisions within the groups have already been referred to.

d. As a slight variation on the AB series, this EF series contained many plants of a somewhat distinct type of blade color named by the writer *"light stipple"* which are not easily confused with the type called stippled "dusky." These "light stipples" appear like ordinary dark-eyed whites or creams whose eye color has become lightly scattered out over the blade. The parallelism to the less involved AB series allows the tracing of an analogy (homology is probably the proper word) between these two series. In individual F_3 families it was perfectly evident that there was the "homologue" of type I, full-colored and eye with no whitish or light area bounding it; there was the homologue of type II, slightly lighter than I but with the eye invariably possessing whitish or lighter-colored areas at its edge as in type II of the AB series (see plate 1); type III was represented in similar "duskies" that were heavily stippled; and then *just where in the AB series we look for clear-bladed dark-eyed forms (type IV) we find here, as a rule, the lightly stippled form;* the parallelism is so close in its many features that the author has not hesitated to group these "light stipples" (which are sharply distinct from the stippled "duskies") with the relatively few clear-bladed dark-eyed plants that occurred, *as type IV,* always stating, however, under IV which were "light stipples" and which were clear-bladed. It would seem, too, that plants passing as clear-bladed may be genotypically "light stipples," as was obviously the case with $(EF)_{255.6}$ (this was parent No. 74 in table 11); its F_3 progeny that developed color were nearly all "light stipples." The whole tendency seems to be to substitute the dark-eyed types with lightly stippled blades for the type (dark-eyed and clear-bladed) that exclusively characterized the AB series. It is questionable whether any of the plants classified as "clear-blades" in the EF series are genotypically so; in table 11 "light stipples" and "clear blades" were separated as well as mere inspection could do this but they are put together under the general heading IV; together they are considered as equivalent to the group of the same number in table 6 and to the type of the same number pictured in plate 1. The belief was expressed above that there must be one or more uninvestigated factors working to shift all the types of blade color in the EF series toward the greater intensity they show as compared to their AB homologues; it is thought that the same uninvestigated factors also stimulate the clear-bladed forms to the greater degree of coloration evidenced in the light stippling out on the flower blade. The point to be emphasized under "d" is the apparent replacement of the clear-bladed form by the "light-stipple" form.

e. Considering the slight differences already discussed as unimportant from the present standpoint, table 11 is seen to offer a decided confirmation of a feature of table 6 that was important from the theoretical point of view: *the paler self-colored types of blade color never throw the (heavily) stippled "dusky" types and the stippled "dusky" parents never produce the paler self-colored types; the full self-colored types (Ia and Ib) give rise to both the paler self-colored (IIa and IIb) and the stippled "duskies" (IIIa and IIIb)*. The conclusion seems inevitable that (expressed phenotypically)

$$\text{type II} + \text{type III} = \text{type I}.$$

The full-colored selfs, then, are simply interaction effects of the paler-colored selfs and the "duskies"; or, the paler self-colored and the "duskies" can arise by the genetic resolution of the full-colored selfs.

Leaving out of consideration the factors that modify blade color in a very minor way we can interpret the results of crossing varieties E and F in nearly the same manner as was done for the AB series. The smaller proportion of F_2 plants without anthocyanin in the blade (or at most in the EF series merely lightly stippled on) indicates that the F_1 plants were not heterozygous for as many genes prerequisite for anthocyanin formation as in the AB series. It will be recalled that in the latter series the F_2 generation showed an apparent rough approximation to a ratio of 9 with blades containing anthocyanin to 7 with blades lacking it. Actually the ratio was 9:7.15 and this on analysis was shown to be due to two sets of triple complementary genes, each complete set by itself making for one of the two simple blade colors. These sets were $E + A + P$

and $E + A + D$. In the EF series the F_2 generation reveals a ratio of 3 plants with blades showing marked anthocyanin development to every 1.37 lacking this or at most with very light stippling on the blade. This is a rough approximation to a 3:1 ratio pointing to a monomeric difference between blades with anthocyanin and blades without anthocyanin. The plus deviation of the second term of the ratio, however, has a significance here that is similar to that in the AB series. The same genes *specific* for types II and III are apparently present in the EF series but only one other (whether E or A is purely arbitrary as far as our account goes) was differential and so heterozygous in F_1. Let us assume that it was gene E; then two simple blade color types, II and III, are present here, the former due to factors E and P and the latter dependent on factors E and D. All three genes are heterozygous in the F_1 generation. In passing to the F_2 generation P and D, either singly or together, enter 15/16 of the zygotes and so act approximately like a single homozygous gene for blade color of some sort, leaving the other gene E to produce the approximately 3:1 ratio. It can be only *approximately* because in 1/16 of the zygotes neither P nor D will be present, causing a non-anthocyanous blade even though E be present.

A factor which differentiates a "bluer" and a "redder" subgroup of each class must also be supposed in the EF series.

By way of summary, the following are the more important factor differences assumed:

E PARENT	F PARENT
b, absence of bluing factor; gives "red" colors when homozygous.	*B*, bluing factor.
P, same as in the AB series; gene for chromogen of paler self-colored type, IIb.	*p*, allelomorph of *P*.
D, as in AB series; chromogen factor for "dusky."	*d*, allelomorph of *D*.
E, as in AB series; chromogen factor for enzyme, supposedly.	*e*, allelomorph of *E*.

Table 12 lists the possible combinations of these factors, the expected numbers for each combination in every 256 individuals, together with the phenotype which each combination produces. Table 13 gives the theoretical expectations recalculated for 530 individuals, and places them beside the observed numbers.

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No.	C des	IIIb Purple	IVa Violet "Stipple"	IVa Violet "Clear blade"	IVb Purple "Stipple"	IVb Purple "Clear blade"	V No antho-cyanin White	V No antho-cyanin Cream	Total
1	(E	—	—	—	—	—	—	—	17
2	(E	—	—	—	—	—	—	—	38
3	(E	—	3	—	1	—	—	7	22
4	(E	1	—	—	—	—	—	—	19
5	(E	1	1	—	—	—	—	—	28
6	(E	—	2	—	2	—	10	3	53
7	(E	—	—	—	—	—	19	—	51
8	(E	3	—	5	—	2	—	25	101
9	(E	2	2	—	—	—	15	—	61
10	(E	1	—	—	—	—	—	5	11
11	(E	—	2	—	—	—	3	1	27
12	(E	—	—	—	—	—	—	20	91
13	(E	10	—	—	—	—	20	4	109
14	(E	3	—	—	—	—	14	—	62
15	(E	5	—	—	—	—	—	—	36
16	(E	—	—	—	—	—	1	—	6
17	(E	7	1	—	—	—	15	—	75
18	(E	6	—	1	—	—	18	4	82
19	(E	—	—	—	—	—	2	5	37
20	(E	—	—	—	—	—	23	—	90
21	(E	—	—	—	—	—	13	1	33
22	(E	3	—	—	—	—	—	11	32
23	(E	—	—	—	—	—	3	3	20
24	(E	9	—	—	—	2	13	—	45
25	(E	—	—	—	2	—	—	4	13
26	(E	4	—	—	—	—	—	—	13
27	(E	8	—	—	8	—	—	—	57
28	(E	—	3	2	—	1	—	—	27
29	(E	—	—	—	—	—	—	—	32
30	(E	—	4	3	—	1	1	3	42
31	(E	—	16	—	5	—	—	20	85
32	(E	—	5	4	2	2	8	1	55
33	(E	—	13	1	1	2	—	19	79
34	(E	—	3	—	—	—	2	—	16
35	(E	—	2	—	—	—	—	—	13
36	(E	—	—	—	—	—	—	7	57
37	(E	—	2	5	2	2	—	12	41
38	(E	—	2	1	4	—	12	2	53
39	(E	—	—	—	—	—	2	4	30
40	(E	—	13	—	2	—	—	—	67
41	(E	—	—	—	—	—	—	—	52
42	(E	—	5	—	1	—	—	—	38
43	(E	—	2	—	1	—	—	3	17

* Dr=da

No.	F₂ par Culture designation	IVa Violet "Stipple"	 "Clear blade"	IVb Purple "Stipple"	 "Clear blade"	V No antho-cyanin White	 Cream	Total
44	$(EF)_{234.8}$	—	—	2	—	—	—	12
45	$(EF)_{233.2}$	—	—	10	—	34	—	100
46	$(EF)_{235.24}$	—	—	19	1	18	5	94
47	$(EF)_{235.29}$	—	—	2	6	7	1	39
48	$(EF)_{233.26}$	—	—	18	4	6	2	64
49	$(EF)_{235.28}$	—	—	9	—	—	—	32
50	$(EF)_{233.5}$	—	—	12	—	10	3	50
51	$(EF)_{234.8}$	—	—	—	—	5	—	27
52	$(EF)_{235.12}$	—	—	—	—	1	1	8
53	$(EF)_{235.56}$	—	—	—	—	—	—	1
54	$(EF)_{235.57}$	—	—	—	—	2	—	2
55	$(EF)_{235.1\frac{1}{2}}$	10	—	—	—	10	9	64
56	$(EF)_{235.22}$	2	—	2	—	—	—	15
57	$(EF)_{235.17}$	3	—	—	—	2	—	6
58	$(EF)_{234.14}$	4	—	—	—	—	4	21
59	$(EF)_{234.9}$	—	—	—	—	—	—	50
60	$(EF)_{235.13}$	—	—	—	—	10	1	28
61	$(EF)_{234.13}$	—	—	—	—	—	—	38
62	$(EF)_{234.15}$	—	—	11	1	—	23	82
63	$(EF)_{234.16}$	—	—	—	—	5	4	44
81	$(EF)_{234.20}$	—	—	—	—	—	—	5
64	$(EF)_{235.5}$	25	—	7	—	—	—	32
65	$(EF)_{233.7}$	7	—	—	—	—	—	7
66	$(EF)_{235.18}$	10	—	9	—	7	—	26
67	$(EF)_{235.10}$	54	1	—	—	—	9	64
68	$(EF)_{235.11}$	42	—	9	—	—	—	51
69	$(EF)_{233.15}$	16	—	—	—	—	—	16
70	$(EF)_{235.15}$	7	—	3	—	—	—	13
73	$(EF)_{234.11}$	15	23	12	3	10	—	63
71	$(EF)_{233.14}$	—	—	45	—	—	—	45
72	$(EF)_{233.46}$	—	—	25	1	—	7	33
74	$(EF)_{233.6}$	—	—	49	3	17	—	69
75	$(EF)_{233.14}$	—	—	7	12	5	2	26
76	$(EF)_{234.17}$	—	—	11	23	—	12	46
77	$(EF)_{234.14}$	—	—	—	—	21	—	21
78	$(EF)_{233.1}$	—	—	—	—	43	—	43
79	$(EF)_{234.15}$	—	—	—	—	—	18	18
80	$(EF)_{233.20}$	—	—	—	—	—	45	45

Total = 3333

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* Dr=darker; M=

† The record of 19ght stipple."

§ These three const

TABLE 12

The possible combinations of the chief factors in the F_2 generation of the EF series.

Factor combinations				Phenotypic formulae	Phenotype	Theoretical numbers
P	*E*	*D*	*B*	*PEDB*	Ia	81
			b	*PEDb*	Ib	27
		d	*B*	*PEdB*	IIa	27
			b	*PEdb*	IIb	9
	e	*D*	*B*	*PeDB*	V	27
			b	*PeDb*	V	9
		d	*B*	*PedB*	V	9
			b	*Pedb*	V	3
p	*E*	*D*	*B*	*pEDB*	IIIa	27
			b	*pEDb*	IIIb	9
		d	*B*	*pEdB*	IVa	9
			b	*pEdb*	IVb	3
	e	*D*	*B*	*peDB*	V	9
			b	*peDb*	V	3
		d	*B*	*pedB*	V	3
			b	*pedb*	V	1

It must be admitted that the calculations in table 13 show theory and observation to be not exceedingly close fits. In view of the relationship of "dusky" (III), the paler self-colored (II), and the full-colored (I) types, elsewhere emphasized, it is interesting to note that a deficiency of the violet "duskies" (IIIa) is accompanied by a deficiency of the "bluer" full-colored selfs (Ia) but with an excess of the paler "bluish" selfs (IIa); some single cause affecting adversely the appearance of violet dusky gametes would bring about simultaneously the other two deviations from the expected.

TABLE 13

Actual and theoretical composition of the F_2 generation of the EF series.

F_2 phenotype	Observed frequencies	Theoretical frequencies for 530 plants
Ia	131	167.67
Ib	62	55.89
IIa	73	55.89
IIb	28	18.63
IIIa	43	55.89
IIIb	27	18.63
IVa	28	18.63
IVb	14	6.21
V	124	132.48
Total	530	529.92

Let us once more consider the ratio of "blues" to "reds" (i.e., any "a" subgroup to its "b" mate),—this time in both tables 5 and 10; and of the ratio of "blues" to "reds" among the progeny of heterozygous "a" parents for the main groups of the F_3 generation of the EF series (as summarized in table 11). It becomes evident that the ratio is not 3:1 as expected on our assumption but averages again about 2.3:1. This may be due, as remarked above, to some sort of selective elimination of the "blue" zygotes; but of the "blues" which survive the author cannot assert that they are weaker than the "reds." The theory of linkage has been invoked already to explain this ratio, by assuming that instead of a single factor, B, conditioning the "blue" colors of the classes, there are two linked genes, B_1 and B_2, the presence of both of which simultaneously is necessary for "blue" formation; and by supposing that the linkage value is 90 percent. The theoretical ratios first proposed for the AB and EF series (see tables 8 and 13) were based on a single dominant factor; those calculations and the numbers actually observed in the experiments in both series are repeated in table 14, together with the amended theoretical ratios, these last being based on the *pair of factors* for "bluing" just discussed. In the EF series the square of chi (χ^2) is much reduced by the bifactorial hypothesis for "bluing," but the value of P remains still very small.

TABLE 14

A comparison of two hypotheses concerning the relation of the bluish to the reddish subgroup.

Type	AB series			EF series		
	Observation	Theoretical 1 B gene	Theoretical 2 B genes	Observation	Theoretical 1 B gene	Theoretical 2 B genes
Ia	76	74.51	69.54	131	167.67	156.49
Ib	33	24.83	29.80	62	55.89	66.07
IIa	26	24.83	23.18	73	55.89	52.16
IIb	13	8.27	9.93	28	18.63	22.36
IIIa	19	24.83	23.18	43	55.89	52.16
IIIb	8	8.27	9.93	27	18.63	22.36
IVa	7	8.27	7.73	28	18.63	17.38
IVb	2	2.75	3.31	14	6.21	7.46
V	130	137.37	137.37	124	132.48	132.48
	314	$\chi^2 = 7.6$ P = .47	$\chi^2 = 4.3$ P = .82	530	$\chi^2 = 40$ P less than .01	$\chi^2 = 29$ P also very small

At the present stage of investigation it is not meant to urge this bifactorial hypothesis for bluing; it is offered simply as a suggestion. The obtaining of "blues" from matings of extracted "reds" would offer substantiation of the hypothesis, as already pointed out; there should be corroboration also in those F_3 families descended from heterozygous (F_2) parents of the Ia, IIa, IIIa, and IVa groups; some of these parents (about one in every eleven) should be heterozygous in just one of the pair of linked bluing factors due to their holding one crossover and one non-crossover chromosome, and such should furnish about 25 percent of "red" progeny; those heterozygous for the two should give 29 or 30 percent of "reds." The percentage of "reds" in the F_3 families of the large EF series has been calculated and the data form the frequency polygon shown in figure 15 (families comprising less than 15 colored individuals are omitted). There is some evidence of a peak at about 21 percent (expected 25 percent); the high peak at about 29 percent is as expected. Further experimental data are needed to settle the matter.

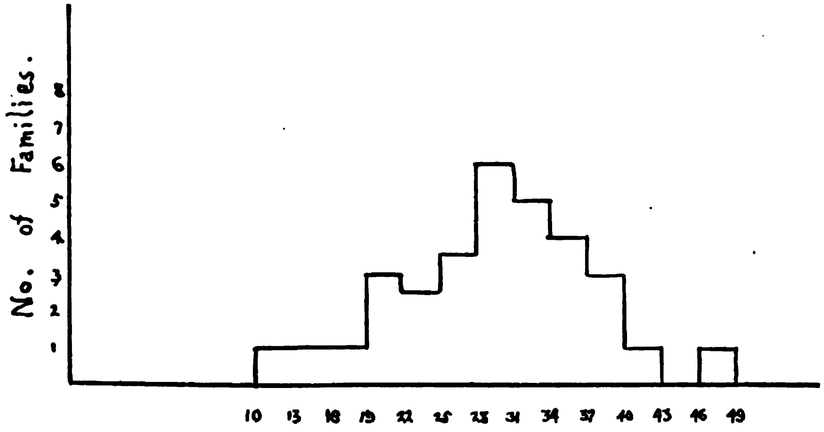


FIGURE 15.—Abscissal numbers show proportion in percent of each F_3 family that is reddish; ordinates give number of families for various abscissal values.

THE CROSSING OF WHITE-EYED PINK (J) AND COMPLETELY WHITE (A)— THE JA SERIES

The J parent was one of 26 sibs resulting from the guarded pollination of a plant of commercial origin; all 26 were similar to one another. Each flower had a white eye as a center, surrounded on the outer blade by a color that was close to the eosin pink of RIDGWAY's guide. The white parent was one of a line that had been observed in pedigree culture for two seasons and had been found to be giving only whites.

The particular pair of plants used as parents were crossed reciprocally giving five similar offspring; the color of the flower of these F_1 hybrids was a complete surprise; they were dark-eyed and the outer blade was one of the full colors, tyrian rose. Three of the plants furnished seed for an F_2 generation of 217 plants. Nine types of flower actually occurred among the descendants of the hybrids of J and A, but only eight of these were recorded as occurring in the F_2 generation. Plate 2 shows in

TABLE 15

Flower type in the F_2 generation of the JA series with their observed and calculated frequencies.

Type	Color of type	Observed numbers in F_2	Theoretical numbers for F_2
Ia	Dark-eyed; blade tyrian rose or bright pomegranate purple; like F_1	64	68.6
Ib	White-eyed; blade between tyrian rose and tyrian pink	20	22.8
IIa	Dark-eyed; blade like next type (IIb) but a trifle darker	34	22.8
IIb	White-eyed; blade about eosin pink; like J parent	13	7.6
IIIa	Dark-eyed; blade about Aster purple or magenta (= non-stippled "dusky")	23	22.8
IIIb	White-eyed; blade near mallow pink and stippled	9	7.6
IV	Completely white; like A parent	48	54.2
Va	Dark-eyed; blade about deep rose pink	6	7.6
Vb	White-eyed; color usually confined to small single arrow-shaped spot on each corolla lobe	0	2.5
	Total	217	216.5

NOTE.—In tables 15 and 16 the distinction between any "a" group and its corresponding "b" group is *not* as in the EF and AB series, a matter of a bluing factor; in the JA series any "a" group is distinguished from its "b" by a factor for dark-eye; this point will be presently discussed. By ELDERTON's tables, P for the data above is about .10.

colors the types of the parents, of the F_1 generation and of the F_2 generation; Type Vb, not reported among the F_2 experimental results, is pictured among the colored F_2 types, since it is believed that because of the exceedingly small amount of color, even when well developed, and of the fluctuability of what little there is (potentially), that Vb *may* have occurred and was counted among the completely white; by the hypothesis to be developed, however, it is expected in only .8 of one percent of the cases and it may not have occurred at all because of the small number of experimental plants, 217. Table 15 summarizes all of the F_2 data.

Within the more deeply colored of the groups some quite minor variations in the depth of pigmentation were in evidence; these were not selected as the basis for further division, since F_3 results showed that they continued of minor importance. The types as given in table 15 were sharply distinct except sometimes in the following cases: light forms of type IIa very much resembled Va plants; a slight bluish tinge in the latter which increased with the aging of the flowers would usually separate doubtful cases, but even this did not prevent one obvious misclassification: $(JA)_{11.91}$ was given as Va in 1918 but by progeny test in 1919 it was shown to have been a lighter IIa plant. In type Vb color is confined as a rule to a single small arrow-shaped spot on each lobe (see plate 2); the color is light and often barely perceptible when it may be easily overlooked and the flower rated as completely white.

During the summer of 1919 thirty-two F_3 families were grown, comprising a total of 1518 individuals. Table 16 presents a summary of data, giving to the left the phenotype of the F_2 parent and in the body of the table the character of the (F_3) offspring.

The following points of F_2 and F_3 generations (as summarized in tables 15 and 16) should be emphasized:

a. The white-eyed condition was recessive in the F_1 generation and reappeared in about one-fourth of the F_2 progeny; the white-eyed F_2 plants gave only white-eyed offspring in the F_3 generation.

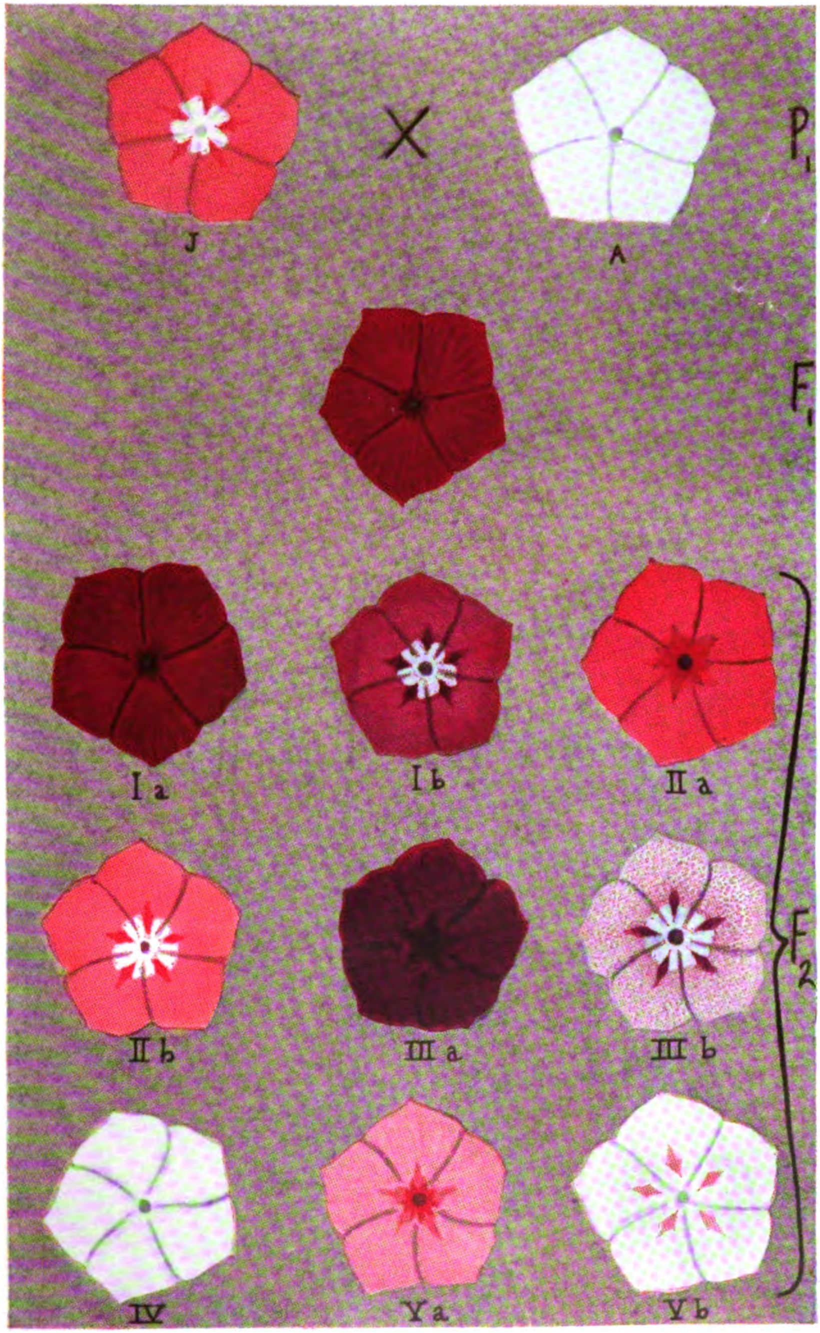
b. The lightest-colored types, Va and Vb, constitute a minor system of colors independent of the rest of the color system, and present in cryptomeric condition probably with all the other colors; at least, one of the factors for the formation of this pale color appears so. Types Va and Vb are hypostatic to all the other color types and it is possible for all of them to throw Va and Vb, as shown in the F_3 results.

c. Unexpected and especially noteworthy is the fact that the main types, I, II, and III, apparently hold to each other the same relationship as do

Legend for plate 2

Types of flower color appearing in the JA series. Designated by J and A at the top are the parents that were crossed. Immediately below J and A is shown their F_1 hybrid; and below the latter the color types appearing in the F_2 and F_3 generations.

KELLY, J. P., FLOWER COLOR IN *PHLOX DRUMMONDII* PLATE 2



the correspondingly designated types of the AB series and EF series. This means that the ***non-stippled*** (self-) magenta or Aster purple type,

TABLE 16

Complete summary of data of F_3 families in the JA series.

F₂ parent, 1918			F₃ progeny, 1919									
No.	Culture designation	Phenotype	Ia	Ib	IIa	IIb	IIIa	IIIb	IV	Va	Vb	Totals
1	$(JA)_{11.62}$	Ia	19	7	10	4	8	2	—	1	1	52
2	$(JA)_{12.74}$	Ia	42	—	11	—	11	—	16	—	—	80
3	$(JA)_{11.73}$	Ia	32	—	12	—	16	—	—	—	—	60
4	$(JA)_{11.8}$	Ia	23	15	9	3	7	4	—	—	1	62
5	$(JA)_{12.64}$	Ia	48	—	—	—	18	—	18	—	—	84
6	$(JA)_{12.72}$	Ia	41	18	8	3	18	5	—	3	3	99
7	$(JA)_{11.22}$	Ib	—	48	—	8	—	—	—	—	—	56
8	$(AJ)_{102.8}$	Ib	—	33	—	4	—	7	12	—	1	57
9	$(JA)_{11.36}$	Ib	—	22	—	—	—	7	9	—	1	39
10	$(JA)_{11.15}$	Ib	—	15	—	8	—	6	20	—	2	51
11	$(JA)_{11.5}$	IIa	—	—	4	1	—	—	6	—	—	11
12	$(JA)_{11.16}$	IIa	—	—	22	11	—	—	—	—	—	33
13	$(JA)_{12.26}$	IIa	—	—	19	6	—	—	13	4	3	45
14	$(AJ)_{102.2}$	IIa	—	—	11	5	—	—	5	—	—	21
15	$(JA)_{11.21}$	IIa	—	—	8	3	—	—	—	2	1	14
16	$(JA)_{12.11}$	IIa	—	—	2	4	—	—	2	—	—	8
17	$(JA)_{12.75}$	IIa	—	—	31	13	—	—	1	8	—	53
18	$(JA)_{12.4}$	IIa	—	—	26	8	—	—	6	—	—	40
19	$(JA)_{12.2}$	IIb	—	—	—	78	—	—	17	—	—	95
20	$(JA)_{11.3}$	IIb	—	—	—	10	—	—	8	—	—	18
21	$(JA)_{12.80}$	IIb	—	—	—	11	—	—	3	—	8	22
22	$(JA)_{11.75}$	IIIa	—	—	—	—	31	12	11	—	—	54
23	$(JA)_{11.79}$	IIIa	—	—	—	—	19	7	15	8	2	51
24	$(JA)_{11.18}$	IIIa	—	—	—	—	28	8	12	5	2	55
25	$(JA)_{11.80}$	IIIa	—	—	—	—	34	—	8	—	—	42
26	$(JA)_{11.67}$	IIIa	—	—	—	—	39	15	—	—	—	54
27	$(JA)_{12.42}$	IIIb	—	—	—	—	—	47	—	—	6	53
28	$(JA)_{11.33}$	IIIb	—	—	—	—	—	15	7	—	1	23
29	$(JA)_{12.51}$	IIIb	—	—	—	—	—	26	9	—	3	38
30	$(JA)_{11.28}$	Va	—	—	—	—	—	—	8	20	11	39
31	$(JA)_{11.30}$	Va	—	—	—	—	—	—	—	52	16	68
32	$(JA)_{11.91}$	Va(?)	—	—	24	4	—	—	1	8	4	41
											Total	1518

IIIa, is equivalent to the *stippled* "dusky" type hitherto designated IIIa; a comparison was made of the living specimens of the JA and EF IIIa types and ignoring the stippled distribution of the latter, one would pronounce the two to be the same; it is interesting to note that the "b" member of the III pair (distinguished especially by white eye instead of dark eye) shows its color more or less stippled. It should be said, too, that the type of EF duskies to which this JA type IIIa corresponds *is the more "reddish" of the "duskies";* it was not at all like the more "bluish" or violet member of the pair of EF "duskies"; the JA type III looks "blue" compared to its associates but we must evidently not attribute this condition to a bluing gene. In the JA series, also, type III never contains II, nor does type II contain III, as is evidenced by the experimental data in table 16. Type I contains both II and III, however, and is considered to be the interaction effects of these. These results were unexpected, since the color of type II of the JA series is in quality different from that of the AB and EF series.

With these observations in mind it is not difficult to construct a factorial scheme to interpret the results:

A PARENT	J PARENT
D, same as in the AB and EF series.	*d,* absence or allelomorph of *D.*
r, absence or allelomorph of *R.*	*R,* factor for chromogen leading to the reddish pink anthocyanin of *J.*
e, absence or allelomorph of *E.*	*E,* factor for enzyme capable of converting chromogens into anthocyanin.
M, factor determining dark eye color; also an intensifier of blade color.	*m,* absence or allelomorph of *M;* in homozygous condition *m* determines the white-eyed condition.

Table 17 lists the possible factorial combinations for this scheme, with the phenotype of each combination and the number of times it is expected to occur in every 256 individuals. The last column of table 15 lists alongside of the observed figures the numbers to be expected in every 217 individuals; a comparison of the observed and calculated numbers shows that the correspondence between theory and fact is fairly close.

TABLE 17

The possible factor combinations for the F_2 generation of the JA series.

Factor combinations				Phenotypic formulae	Phenotype	Theoretical numbers
E	*D*	*R*	*M*	*EDRM*	Ia	81
			m	*EDRm*	Ib	27
		r	*M*	*EDrM*	IIIa	27
			m	*EDrm*	IIIb	9
	d	*R*	*M*	*EdRM*	IIa	27
			m	*EdRm*	IIb	9
		r	*M*	*EdrM*	Va	9
			m	*Edrm*	Vb	3
e	*D*	*R*	*M*	*eDRM*	IV	27
			m	*eDRm*	IV	9
		r	*M*	*eDrM*	IV	9
			m	*eDrm*	IV	3
	d	*R*	*M*	*edRM*	IV	9
			m	*edRm*	IV	3
		r	*M*	*edrM*	IV	3
			m	*edrm*	IV	1

Total 256

GILBERT (1915) briefly described the results of three hybridizations in *Phlox Drummondii* in all of which a variety called "Carnea" was the pollen parent. From the colored plate accompanying his article it is obvious that his "Carnea" is like the J strain of my cultures; his seed parents were different, none of them bearing completely white flowers. One of his crossings gave F_2 results that make interesting comparison with the JA data presented above. He crossed his "Carnea" to a variety he says was known commercially as "Coccinea." This is described and pictured as bluish lilac with a dark eye. His plate D shows it to closely resemble the type IIIa ("dusky") already described. His F_1 flowers were what would be expected if my J or type IIb and "dusky" were crossed, i.e., a brilliant carmine, and resembling the F_1 in my JA series. GILBERT's F_2 generation of 115 plants comprised seven types; his plate would seem to prove that five of these types closely corresponded to or were identical with the types Ia, Ib, IIa, IIb, and IIIa of this paper. A sixth type with formula in the text including factors *"ee"* (= white eye) and *described as white,* was *pictured* as a dark-eyed white. His seventh type is described and pictured as a dark-eyed white and was reported once in the 115 plants. Assuming that his sixth type was as his text described, GILBERT secured six of the nine types which are here reported for the JA series. The total absence in GILBERT's cultures of a type like IIIb is surprising as is also the fact that the faintly colored independent types were not reported.

To interpret these results GILBERT supposed three factors necessary (with their recessive allelomorphs), (1) a factor for dark eye, (2) a factor for blue pigment, and (3) a distinct and independent "red" factor carried by the "Carnea" (= J) parent; these factors, in general, correspond to the writer's *M, D,* and *P,* respectively. GILBERT did not expressly state that the bright carmine was an interaction product of the factors for his dusky and reddish-pink sorts, but his genetic formulae imply this.

Bright carmine,	*EEBBRR*	(corresponds to my type	Ia)
Reddish-pink,	*EEbbRR*	(" " " "	IIa)
Bluish-lilac,	*EEBBrr*	(" " " "	IIIa)

GILBERT carried none of his crosses to the F_8 generation so that he could not know from actual breeding that his reddish-pink never carried the gene for bluish-lilac, nor that the bluish-lilac never threw the reddish-pink, and that the heterozygous carmines could give both in the next generation thus pointing to the reddish-pink and bluish-lilac as resolution products.

In another of GILBERT's crosses, he records an unexpected *preponderance of white* "which is unaccounted for." An explanation of this condition has been presented above in the exposition of the results of the AB series.

SOME SIMPLER CASES OF COLOR INHERITANCE IN PHLOX DRUMMONDII

The crossing of white-eyed tyrian rose (*P*) *and white-eyed pink* (*J*)

The line to which the P parent belonged has been carried on in pedigree culture in small numbers from season to season. No deviations from type have been noticed. In this P strain the flowers are white-eyed with outer blade about a brilliant tyrian rose. The J type has already been described and pictured. The two individuals used as parents were crossed reciprocally giving seed for an F_1 generation of 12 plants; the flowers of the F_1 generation were all indistinguishable from those of the inbred P strain.

Three of the F_1 plants furnished seed for an F_2 generation of 218 plants grown in 1918. The F_2 group presented a sharp segregation into the two parental types, there having been 165 P type to 53 J type. On a simple unifactorial basis expectation calls for 163.5 P type to 54.5 J type. The correspondence, therefore, is very close.

By the conventional manner of Mendelian interpretation these experimental results would lead to the statement, probably, that we have obtained a definite idea of the specific difference between P and J, that a factor in the J parent conditioning the reddish-pink J coloration is allelomorphic to a different factor in the P parent making for the carmine color. It would seem that the matter is not so simple as it appears, however. The segregation, of course, indicates a difference of some kind, but gives us no idea of the nature of the difference. The evidence on which this assertion is based was secured in the JA series; the P type really arose there anew following hybridization and recombination and was designated as type Ib. The P strain was like the slightly darker variants of type Ib in the JA series. We learned there that Ib (and, hence, supposedly, P) is due to the composite or complementary effects of the factors for J (= IIb) and for the stippled mallow pink (= IIIb); that is P = J (IIb) + IIIb. We inferred also that J never carries IIIb; and that the types that correspond to P and J *both* carry the gene for the J pigmentation. The hybrid between P and J must have been, therefore, homozygous for the factor conditioning J, and the segregation of J in 25 percent of the F_2 in this JP series does not point to the real difference between J and P. The real difference between P and J, which is the presence of the complementary gene for mallow coloration, i.e., for type IIIb (present in P and lacking in J), we know from our previous results in the JA series, but would probably have never even suspected from the results of crossing J and P. All gametes of the F_1 generation (in the JP series), then, contained the factors for producing the J type of color; half of these in addition held the factor for the IIIb type of coloration revealed in the JA series and half lacked the latter; all F_2 zygotes of the P type must have carried the gene for IIIb color and all J zygotes presumably lacked this factor. We arrive thus at a seemingly paradoxical position: the 53 J segregates of the F_2 generation indicated a feature in which the parents were similar and the real difference was masked, for wherever the really differentiating factor for the mallow pink color appeared it was compelled to give a full-colored interaction product, because of the homozygous condition of the factor for the J color; and the absence or allelomorph of this factor for mallow-pink would allow the unchanged J color to appear.

The results of crossing P and J, therefore, pointed to a unifactorial difference but, in themselves, really furnished no clue as to the real nature of the difference.

The genetic relationship of white (A) and cream (F)

Both parents were from pedigree lines grown in considerable numbers during several seasons and were known to be breeding true—the former, strain A, for completely white flowers, and the latter, strain F, for completely cream-colored flowers. Various members of the two types or strains were crossed giving seed for an F_1 generation of 47 plants; all F_1 hybrids were completely white and indistinguishable from the A parent. Reciprocal hybrids were included but the F_1 plants were white no matter whether the seed parent was white or cream.

Seven of the F_1 individuals provided seed for an F_2 generation of 169 plants; the group presented a simple segregation into 132 white and 37 cream. The expectation for a monomeric difference in the case of 169 individuals is 126.75 : 42.25. The fit of the theoretical to the actual is close enough to warrant postulating a single factorial difference operating to distinguish the white from the cream.

The genetic relationship of white with dark eye (B) and cream without dark eye (F)

Various individuals of both types were crossed reciprocally. The parents were from lines which have been for several seasons in pedigree culture and known to be true to their respective types. Reciprocal hybrids were identical. A total of 67 F_1 hybrids bloomed during 1917 and 1918, and all were similar, bearing white flowers with dark eyes, resembling the B parents.

Several F_1 plants gave seed for an F_2 generation of 213 individuals. Four sharply distinct types appeared among these F_2 plants, the types and their numbers being given in table 18.

TABLE 18

Actual and theoretical composition of the F_2 generaton from the cross of B and F.

F_2 types	Observed numbers	Calculated numbers
Dark-eyed white	112	119.8
Dark-eyed cream	37	39.9
Completely white	47	39.9
Completely cream	17	13.3
Total	213	212.9

Obviously two independent factorial differences are at work in this case, the factor W, a dominant one, determining the white color of the plastids; its allelomorph, w, when homozygous, causes the cream color of the plastids. The other factor has already been encountered in the JA series, i.e., M, which causes the appearance of an anthocyanin eye at the centre of the flower; its allelomorph, m, is responsible for the colorless condition at the centre. In the last column of the table the expectations on such a basis are entered.

CONCLUSIONS AND SUMMARY

1. Since the introduction of *Phlox Drummondii* into culture less than 85 years ago a large number of marked variations has occurred, offering good opportunities for the genetical analysis of the species.

2. The distinguishing features of the several varieties upon which the present report is based, and their cultural designations (not to be confused with the factorial symbols) are as follows:

Cuspidate flowers	L
Funnel-shaped flowers	I
Completely white flowers	A
Dark-eyed white flowers	B
Dark-eyed brilliant carmine or pomegranate purple flowers	E
Completely cream flowers	F
White-eyed reddish pink	J
White-eyed tyrian rose	P

3. An hereditary variation named var. *cuspidata* has arisen with much reduced corolla limb bearing on each lobe of the corolla two short lateral teeth and one long median tooth. A difference of a single gene distinguishes the entire-petalled from this cuspidate-petalled kind. An independent factor or factors (for breadth of lobes) interacts with the *cuspidata* and alters the character of the side teeth.

The *fimbriata* "variety" is merely a simple monoheterozygote of the entire and *cuspidata* types; it represents the only case of non-dominance for a factor, thus far known in *Phlox Drummondii*. The hybrid nature of *fimbriata* necessitates certain taxonomic amendments, indicated in the presentation.

The *cuspidata* gene would seem to act (chiefly) to decrease the rate of epidermal growth, this inhibition being weaker in three locations. The factorial symbol I_6 is suggested to designate this factor.

4. Another hereditary variation in the form of the flower is represented by the funnel-shaped phloxes; a single factorial difference distinguishes the funnel-shaped from the salver-shaped condition normal for the Phlox genus. The factor *S* is presumed to determine the salver shape and its allelomorph *s*, which is completely recessive, the funnel shape. Two other grades of corolla expansion were found. The factor *s* itself or some other uninvestigated gene closely linked with it, causes the colored funnel-shaped flowers to appear white-streaked.

5. There are pairs or sets of complementary factors in *Phlox Drummondii*. In some cases in addition to the compound or interacting effect, the individual members of the pairs or sets are capable of separate and distinct somatic expression. In other cases each pair or set of genes becomes evident only in the single compound character, the components remaining latent (invisible or cryptomeric) when not present together.

a. The crossing of the strains A and B (in both of which the flower blades are white) and of A and J (the former with white blade and the latter with light pink) gave in each case a surprisingly different full-colored F_1 type.

b. The simultaneous presence of three factors is necessary before a simple blade color can develop; the F_1 hybrid of A and B proved heterozygous for all three of these necessary factors as evidenced by the large proportion of F_2 white-bladed plants and the character of the families from F_2 colored plants. The three factors are conceived to be, first, one, *P*, that leads to chromogen formation; secondly, one *E*, that leads to the production of an enzyme; and lastly one that leads to the production of an activator of the enzyme, *A*.

c. The simple type of blade color caused by the presence of the minimum complement of the three necessary factors varies, depending presumably on the character of the chromogen base present. One (type IIb of the AB and EF series) is thought to be due to the simultaneous presence of genes *E* and *A*, and the chromogen-producing gene *P*. A second simple type of blade color, the "dusky," both stippled and unstippled, is supposed to be due to the same factors, *E* and *A*, acting on a different chromogen due to the factor *D*. A third of the simple types is considered due to the factors *E* and *A* acting on a chromogen *R*.

d. The F_2 and F_3 data led to the following inferences: Blade color type due to $P\ E\ A$ + type due to $D\ E\ A$ = a distinct full-colored type designated Ia in the account of the AB and EF series and in plate 1. Ia is considered to be due merely to the interaction of factors P and D in the presence of E and A; no factors that may be called specific for the Ia type are in play. Similarly, the type due to factors $R\ E\ A$ + the type due to $D\ E\ A$ give a full-colored type designated Ia in the JA series and in plate 2. These generalizations are based on the character of the F_3 results; the critical test, the actual putting together of the simple types by hybridization has not been accomplished.

6. Modifying factors have been in evidence:

a. The AB and EF series of plants exhibited a bluing factor (or linked pair of factors(?)), independent of the essential color producers, which doubled the number of the color groups; for every "reddish" group it made a corresponding more "bluish" group.

b. A simple dominant diluting factor of minor effect occurred in the EF series,—designated D_i.

c. A minor factor, slightly reddening and intensifying simultaneously was referred to briefly in the EF series,—designated D_e.

d. The dark-eye factor, M, led to intensification of the blade color.

7. Dark-eyed forms differ from the light-eyed forms by a single factorial difference,—designated M. Its allelomorph causes the white eye.

8. The cream-colored condition is a simple recessive to the white-colored; gene W determines whiteness and its allelomorph, w, causes the cream. The same relationship of white to cream is inferred from GILBERT's published results.

9. The chromosome number in *Phlox Drummondii* is 14 for the cells of the root-tip.

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BEARING ON HEREDITY AND
VARIATION

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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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STUDIES IN MILK SECRETION. VI. ON THE VARIATIONS AND CORRELATIONS OF BUTTER-FAT PERCENTAGE WITH AGE IN JERSEY CATTLE[1]

JOHN W. GOWEN

Maine Agricultural Experiment Station, Orono, Maine

[Received September 8, 1919]

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[1] Papers from the Biological Laboratory, MAINE AGRICULTURAL EXPERIMENT STATION, No. 127.

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INTRODUCTION

The problems of milk secretion taken from their economic and scientific aspects may be said to be two-fold, the first phase of the subject dealing with the problems connected with the production of the quantity of milk, the second phase considering the quality or amount of the constituents per unit volume of the milk. This second phase to the minds of most people has come to mean for milk production the amount of butter fat per unit volume of the milk or the percentage of this butter fat.

In the preceding paper of this series (GOWEN 1920) the subject of the variations and correlations of milk secretion with age was examined analytically. In this paper it is proposed to deal with the normal fluctuations and associations of the butter-fat percentage for the milk of the same cows used in the preceding study.

The theorem chosen is a small part of that greater problem which has come to be known under the title of developmental mechanics. If a group of like animals are measured for any character and the measurements are brought together in a curve representing the individuals in the group, the position of any individual in the curve and the shape of the curve itself are the functions of the two basic variables, environment and heredity, given such prominence by the work of GALTON (1889, 1895). This environment may play a larger or a smaller part in its influence on the character. In most inheritance studies of what might be called qualitative characters, commonly classified as the chemist classifies in analyzing a chemical compound for its constituent elements, as barium present or barium absent, the environmental differences cause little variation in the somatic appearance of the character. In other words, put in quantitative terms the coefficient of variability of the character is low or as the physicist says the character is constant. The place of the individual in a curve then is due largely, if not entirely, to heredity.

In the so-called quantitative characters the conditions are reversed to a certain extent. The superimposed variability of the conditions under which the organisms exist plays its part along with heredity in determining the place that the individual will take in the variation curve. Clearly in a study of the hereditary nature of such a quantitative character a knowledge of its variation is essential to any adequate study of the subject. Before the milk production or butter-fat percentage of a heifer and an age cow are compared we must know what has come between, for this may be and often is a part of heredity itself.

What these investigations,—the first on milk production and the present one on butter-fat percentage,—have attempted to do is to analyze the individual variations of the individuals in one curve in terms of their component parts.

MATERIAL AND METHODS

The material and methods used are the same as those of the previous paper (GOWEN 1920) save that certain of the cows kept in the early history of the herd were not tested for butter fat. The number remaining after these were discarded were 1713 with complete eight-months butter-fat percentage records. Throughout this study all of the records are for the first eight months of the lactations that extend at least through the ninth month. For the benefit of those who are unfamiliar with the previous paper, that part of the introduction significant to the data and its manner of collection is quoted.

> "The data are exceptional in the following ways: (1) The records extend back to the year 1897 when the herd was organized; (2) The animals are practically all straight island stock; (3) they have been under the oversight and direction of one manager since 1901; (4) exact records are kept of the milk production, butter fat and butter-fat percent; (5) many of the individual animals have records for several different lactations.
>
> The elimination of variation caused by varying the five factors above in the records of cows to be used for exact analysis of the laws of milk and butter-fat production is important, as it has been often shown that such differences can influence the herd's production. It is obvious that these records are free from such variables. They constitute a homogeneous group of data representing the island Jersey under constant conditions of management and climate.
>
> The data used for study are all from normal healthy cows. Two diseases have been present in the herd, tuberculosis and abortion. The tuberculous animals were all eliminated early in the herd's history by the use of the tuberculin test. All records from animals which were proven to be tubercular or which aborted were not used. Records from animals normally healthy but sick during a given lactation were not used. All of the cows have been kept in climatic conditions similar to those of western Virginia.
>
> A word as to the method of keeping the data and its transfer to this Station. All records are made at the time of milking, on the dairy milk sheet for the given cow, which is kept in the barn. The milking taking place twice a day the records are for night and morning. The weekly production taken from these sheets is transferred to the herd ledger by a trained bookkeeper. The total production for a given month is found together with the yearly production by adding the weekly totals. All records are recorded to pounds and tenths. The cows are tested bi-

monthly by the Babcock test and the percentage of butter fat is recorded beside its corresponding monthly milk yield. All weighings and readings are recorded immediately after they are made so there is little chance of inaccuracy. From these records the author has extracted 1741 complete 8-months records of healthy cows for milk production. Of these 1741, 1713 have records for the butter-fat percent. The weighted monthly averages of the bi-monthly test have been used to obtain the weighted 8-months average for the 8-months lactation period chosen for study."

VARIATION OF BUTTER-FAT PERCENTAGE IN JERSEY MILK WITH THE AGE WHEN THE TEST WAS MADE

The records for the mean butter-fat percentage for the 8 months of lactation have been calculated by the author. These records were formed into frequency distributions in which the class interval was fifteen-hundredths of a percent. Distributions were made for the frequencies of the different tests at the ages two years to three years, three years to four, *et seq.*, up to the eighth year of age. The frequency distributions of the tests made during the ages eight and nine years were grouped into one distribution as were the tests made when the cow was ten years old or older. The data for the separate ages were gathered together to form the last frequency distribution presented. This distribution may be said to represent the distribution of the butter-fat percentage of all ages of pure-bred Jersey cows. Such a distribution has considerable value for the comparison of the records of other breeds and other herds with these dealt with in this paper. Table 1 gives the absolute and percentage frequencies of these data.

Certain tentative conclusions may be drawn from a study of table 1 in conjunction with figure 1 regarding the frequency distributions for butter-fat percentage.

(1) The frequency polygons appear quite symmetrical. One only, at the ages eight to ten years, diverges noticeably from this form. In this respect the distribution of milk production in the previous paper agree with these for butter-fat percentage.

(2) Relative to the total percentage of butter fat produced these distributions do not appear to have a large range. The range itself is at the top of the scale of butter-fat concentration of dairy cows. Thus these cows have as the lowest test 3.65 to 3.80 and as the highest test 6.80 to 6.95, whereas the Holstein-Friesian (Gowen 1920) cows have been shown by the author to vary between the limits 2.4-2.6 to 4.6-4.8 in the mean butter-fat percentage of their year tests. It would seem that

TABLE I

Frequency distribution of variation for the mean eight-months butter-fat percentage of a homogeneous herd of Jersey cows at different ages. Absolute and percentage frequencies.

Percent butter fat	Age 2 yrs. 0 mo. to 3 yrs. 0 mo.		Age 3 yrs. 0 mo. to 4 yrs. 0 mo.		Age 4 yrs. 0 mo. to 5 yrs. 0 mo.		Age 5 yrs. 0 mo. to 6 yrs. 0 mo.		Age 6 yrs. 0 mo. to 7 yrs. 0 mo.	
	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency
3.65-3.80	1	.34	—	—	—	—	—	—	—	—
3.80-3.95	—	—	—	—	—	—	—	—	—	—
3.95-4.10	—	—	—	—	—	—	—	—	1	.52
4.10-4.25	—	—	—	—	—	—	3	1.35	1	.52
4.25-4.40	5	1.69	4	1.63	2	.87	—	—	6	3.12
4.40-4.55	14	4.73	9	3.67	4	1.74	4	1.80	6	3.12
4.55-4.70	13	4.39	14	5.71	11	4.78	16	7.21	12	6.25
4.70-4.85	16	5.41	16	6.53	21	9.13	16	7.21	15	7.81
4.85-5.00	29	0.80	24	9.80	32	13.91	24	10.81	23	11.98
5.00-5.15	42	14.19	31	12.65	27	11.74	31	13.96	28	14.58
5.15-5.30	44	14.86	44	17.96	30	13.04	34	15.32	26	13.54
5.30-5.45	48	16.22	22	8.98	29	12.61	28	12.61	23	11.98
5.45-5.60	28	9.46	25	10.20	21	9.13	27	12.16	21	10.94
5.60-5.75	12	4.05	23	9.39	21	9.13	17	7.66	10	5.21
5.75-5.90	13	4.39	9	3.67	12	5.22	10	4.50	8	4.17
5.90-6.05	13	4.39	11	4.49	10	4.35	8	3.60	5	2.60
6.05-6.20	8	2.70	2	.82	3	1.30	1	.45	4	2.08
6.20-6.35	5	1.69	3	1.22	3	1.30	—	—	1	.52
6.35-6.50	2	.68	3	1.22	1	.43	1	.45	1	.52
6.50-6.65	1	.34	2	.82	2	.87	1	.45	1	.52
6.65-6.80	1	.34	2	.82	—	—	1	.45	—	—
6.80-6.95	1	.34	1	.41	1	.43	—	—	—	—
Total	296	100.01	245	99.99	230	99.98	222	99.99	192	99.98

TABLE 1 (continued)

Frequency distribution of variation for the mean eight-months' butter-fat percentage of a homogeneous herd of Jersey cows at different ages. Absolute and percentage frequencies.

Percent butter fat	Age 7 yrs. 0 mo. to 8 yrs. 0 mo.		Age 8 yrs. 0 mo. to 9 yrs. 0 mo.		Age 9 yrs. 0 mo. to 10 yrs. 0 mo.		Age 10 yrs. 0 mo. and above		Total population	
	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency	Absolute frequency	Percentage frequency
3.65-3.80	—	—	—	—	—	—	—	—	1	.06
3.80-3.95	—	—	1	.79	1	1.18	—	—	2	.12
3.95-4.10	2	1.17	—	—	1	1.18	2	1.87	6	.35
4.10-4.25	1	.58	—	—	1	1.18	2	1.87	8	.47
4.25-4.40	2	1.17	3	2.36	5	5.88	5	4.67	32	1.87
4.40-4.55	7	4.09	8	6.30	2	2.35	2	1.87	57	3.33
4.55-4.70	9	5.26	10	7.87	6	7.06	5	4.67	99	5.78
4.70-4.85	17	9.94	8	6.30	5	5.88	10	9.34	126	7.36
4.85-5.00	22	12.87	10	7.87	9	10.59	11	10.28	187	10.92
5.00-5.15	19	11.11	17	13.39	7	8.24	11	10.28	216	12.61
5.15-5.30	28	16.37	21	16.54	7	8.24	21	19.63	258	15.06
5.30-5.45	28	16.37	18	14.17	14	16.47	14	13.08	231	13.49
5.45-5.60	16	9.36	18	14.17	13	15.29	13	12.15	189	11.03
5.60-5.75	6	3.51	4	3.15	4	4.71	4	3.74	104	6.07
5.75-5.90	2	1.17	3	2.36	7	8.24	3	2.80	71	4.14
5.90-6.05	6	3.51	5	3.94	3	3.53	1	.93	63	3.68
6.05-6.20	5	2.92	1	.79	—	—	2	1.87	27	1.58
6.20-6.35	—	—	—	—	—	—	1	.93	13	.76
6.35-6.50	—	—	—	—	—	—	—	—	8	.47
6.50-6.65	—	—	—	—	—	—	—	—	7	.41
6.65-6.80	—	—	—	—	—	—	—	—	4	.23
6.80-6.95	1	.58	—	—	—	—	—	—	4	.23
Total	171	99.98	127	100.00	85	100.02	107	99.98	1713	100.02

these two tests are the two extremes of the scale for long-time records of dairy cows.

(3) The polygons do not change their shape with increasing age as was the case of the polygons for the milk production.

(4) Each distribution appears to be unimodal although the flat-topped nature of the frequencies around the mode does not make this conclusion entirely certain.

The chief physical constants for these data are presented in table 2. The four constants presented are the mean, standard deviation, coefficient of variation and skewness.

Several features of general interest concerning butter-fat secretion are evidenced by this table. These points can only be touched hurriedly as it is planned to take up most of them individually in later sections of this paper.

The mean butter-fat percentage is the highest in the early ages at which the Jersey cow's mammary gland is functioning. From this high point the percentage of this butter declines irregularly toward the older years of the cow's life. The lowest percentage is reached when the cow is over ten years of age. The difference between the highest mean value of the percentage of butter fat occurring at three years old and the lowest mean value at ten years and older (5.2777 ± .0204 and 5.1339 ± .0288) is 0.1438 ± .0354 or the difference is 4.05 times the probable error. Such a difference while only mediocre, is likely to be significant. The point will be discussed later in connection with other data. The mean percentage of butter fat of these Jersey cows (5.2260 ± .0073) agrees fairly well with that for other pure-bred Jerseys (5.12) published by the author in table 2 of a previous paper (GOWEN 1919). As the data on which the 5.12 percentage was based, included a wide variety of conditions, climate, management, etc., it would appear reasonable to suppose that this figure represents a fair average for the Jersey breed. If such is in fact the case the average production of the Jerseys included in the herd studied are above those of the breed as a whole in butter-fat percentage contained in their milk. The difference is slight in absolute amount, however.

If we examine the butter-fat concentration of the milk of the various breeds summarized in the table referred to above, we see that the Jersey stands at the top of the list of these twenty-eight breeds as to the amount of butter fat produced in its milk. The variation of this mean butter fat is between 3.05 and 5.12 percent. The Jerseys are more than two

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TABLE 2

Constants of variation of butter-fat percent for the successive ages at test in Jersey milk. (8 months lactation period)

Age at test	Mean	Standard deviation	Coefficient of variation	Skewness
2 yrs. 0 mo. to 3 yrs. 0 mo.	5.2635 ± .0183	0.4662 ± .0129	8.8581 ± .3473	+ 0.1333 ± .0490
3 " 0 " " 4 " 0 "	5.2777 ± .0204	0.4749 ± .0145	8.9976 ± .3909	+ 0.2635 ± .0556
4 " 0 " " 5 " 0 "	5.2759 ± .0196	0.4415 ± .0139	8.3680 ± .3744	+ 0.2744 ± .0583
5 " 0 " " 6 " 0 "	5.2345 ± .0187	0.4132 ± .0132	7.8938 ± .3644	———
6 " 0 " " 7 " 0 "	5.1875 ± .0216	0.4445 ± .0153	8.5686 ± .4191	———
7 " 0 " " 8 " 0 "	5.1697 ± .0223	0.4322 ± .0158	8.3599 ± .4359	+ 0.1047 ± .0732
8 " 0 " " 9 " 0 "	5.1553 ± .0249	0.4156 ± .0176	8.0619 ± .4359	— 0.1357 ± .0718
9 " 0 " " 10 " 0 "	5.1668 ± .0353	0.4818 ± .0249	9.3259 ± .6875	— 0.5656 ± .2963
10 " 0 " " above	5.1339 ± .0288	0.4419 ± .0204	8.6073 ± .5647	———
Total population	5.2260 ± .0073	0.4492 ± .0052	8.5950 ± .0995	+ 0.1003 ± .0209

percent greater in mean butter-fat percentage than are the lowest cows of the breed. It is especially instructive to study the distribution of these tests a little more closely. For this purpose the tests were grouped into two-tenths percent intervals. Such a distribution gives some appreciation of the hereditary factors which may be expected to occur in the given breed. The results of such a grouping show that there are two breeds, the Jersey and the Guernsey, at the top of the scale for the butter-fat concentration of their milk. The percentage is around 5 percent. In the other group are included the breeds of cattle with a mean butter-fat percentage around 3.7. Between these two groups there is a distinct break between a mean percentage of 4.2 and 4.6. Such a break is highly suggestive of an hereditary difference of at least one unit between these breeds. In this connection the range or spread of the frequency distributions taken for each of these high-test and low-test groups is of interest. Taking the data from the Jerseys of this paper and the Holstein-Friesians of the above-mentioned paper the range of butter fat for the first is 3.65 to 6.95 while that for the Holstein-Friesians is 2.4 to 4.8. As the two frequencies are not very far from normal and as what skewness there is is plus, it follows that the overlap of these curves constitutes only a small area of the total covered by them. The differences of the two breeds are therefore quite distinct. The differences in the scatter of the two groups is also significant as measured by the standard deviation. The standard deviation for the Holstein-Friesian group is 0.318 ± .004 and that of this Jersey group is 0.449 ± .005 or the difference and the probable error are 0.131 ± .006. Absolutely considered the higher fat testers are more variable than the lower-butter-fat-percentage cows. In the Jersey or highest group no influence of age on the standard deviation appears to exist.

The coefficient of variation is worth especial study as it gives us in comparable terms the relation between the standard deviation of a distribution and its mean. For our problem the conclusions to be derived from it are, unfortunately, not so straight-forward, as we are dealing with the index, butter-fat percentage. Reflection on the purpose of the coefficients of variation will make clear that the use of coefficients of variation comes in ridding the coefficient of the terms in which the data are recorded. In other words the coefficient is made a pure number. This is also just what an index does, consequently the use of a coefficient of variation of an index is somewhat like calculating the variation of a pure number. How much this influences the conclusions to be derived from such coefficient of variation is a matter of some doubt. That there is some influence is known; it is, however, altogether probable that this disturbance is not so great but what some conclusion may be drawn from the calculated coefficients of variation even admitting these disturbances.

The need for such a comparison becomes especially clear in our data on butter-fat percentage. Here the character studied is the concentration of the butter fat in the milk and not the total mass or pounds of this fat secreted for a lactation. Information is desired on the variation of the functioning of the cells which secrete this concentrated fat emulsion in comparison with those of cells of the mammary secreting a low concentration of fat. Furthermore, comparison data for the variation of the ability of other glandular cells in their secretory activity are desirable. For these reasons it has seemed best to present coefficients of variation for such data realizing in so doing that too wide conclusions cannot be drawn from them. The data for this comparison are given in table 3. In this table are also included, to avoid the necessity for an additional table, the calculated skewness of the frequency distributions, as these data will be used shortly.

The standard deviations of the butter-fat percentage of the milk produced by the four breeds, Jersey, Guernsey, Holstein-Friesian and Ayrshire, show a relation to the mean concentration of this butter fat, such that the breeds producing the greatest concentration have a significantly larger variation than do the breeds whose milk contains less fat. The solids other than the butter fat, contained in the milk of the Holstein-Friesian cows, show approximately the same standard deviation as does the butter fat of this breed. Such a low standard deviation in comparison to the mean solids-not-fat percentage of about two and one-half times the mean butter-fat percentage leads to a coefficient of variation of about half the size of that for the butter-fat percentage.

The standard deviations of the percentage constituents of the egg parts are all higher than those for the percentage constituents of the parts of the milk. Thus the stadard deviations of the percentage of albumin in the egg is 2.75, that of the yolk is 2.70 and of the shell 1.04, whereas for the butter-fat percentage the standard deviations range from 0.32 to 0.48. This would seem to indicate a real difference in variability between the functioning of the gland cells of the udder of the cow and the oviduct of the hen. The mean percentage of the different parts of the egg are considerably larger than those of the milk parts, however. For the percentage of yolk and the percentage of shell the coefficients of variation agree well with those found for the variation

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TABLE 3

The variation and amount of asymmetry of the concentration of the components of known secretions.

Character	Mean percentage	Standard deviation	Coefficient of variation	Skewness	Source of data
Jersey butter-fat percentage..	5.22 ± .01	0.45 ± .01	8.60 ± .10	+ 0.10 ± .02	This paper
Holstein-Friesian butter-fat percentage	3.44 ± .01	0.32 ± .00	9.23 ± .12	+ 0.15 ± .02	GOWEN (1919)
Holstein-Friesian solid-not-fat percentage	8.60 ± .01	0.34 ± .01	3.92 ± .11	+ 0.17 ± .05	GOWEN (1919)
Ayrshire butter-fat percentage.	3.68 ± .01	0.32 ± .01	8.76 ± .17*	+ 0.16 ± .03*	VIGOR (1913)
Guernsey butter-fat percentage	5.03 ± .00	0.48 ± .00	9.45 ± .01	+ 0.12 ± .01	GOWEN (1919)
Egg of domestic fowl†:					
Albumen percentage	59.83 ± .04	2.75 ± .03	4.59 ± .03*	+ 0.27 ± .02*	CURTIS (1914)
Yolk percentage	30.00 ± .04	2.70 ± .02	8.99 ± .06*	+ 0.21 ± .02*	CURTIS (1914)
Shell percentage	10.13 ± .01	1.04 ± .01	10.30 ± .06*	+ 0.10 ± .02*	CURTIS (1914)

* These constants were calculated by the author from the data presented by the different investigators whose papers are cited. The means and standard deviations cited from these authors have been checked.

† The author is indebted to Dr. M. R. CURTIS for the loan of the original data on which the calculations were based for the variation of the parts of the egg.

of the butter-fat percentage. The coefficient of variation for the albumin does not agree with that of the butter-fat percentage but does agree with that of the solids-not-fat in the milk of the cow. In the formation of the egg of the domestic fowl it is well known that only certain cells can secrete a given substance. The similar variation of the protein-containing solids-not-fat and the albumin portion of the egg, and the similar variation of the lipin portion of the milk and the lipin portion of the egg, calls attention to the lack of knowledge concerning the exact nature of this secretory activity of the mammary gland and the possibility that there may be two types of cells in this gland, of separate and distinct secreting function.

Returning to table 2, no skewness is present in three of the nine distributions. In the remaining six distributions at the different ages there are four in which the skewness is plus and two in which the skewness is minus. The frequency distributions of butter-fat percentage at the first three years of the lactation life of the Jersey cow are skew in the plus direction. This skewness increases to the fifth year of lactation. At this age the curves for the butter-fat percentages are symmetrical. The minus skewness of the eight-and-nine years of age is quite unlooked for. Negative skewness is on the whole, rare. Why milk production at these ages should change to become minus, and minus to as large an amount in the ninth year of age, is not clear.

The general frequency distribution for the butter-fat percentage of Jersey milk has a plus skewness of rather small amount. The comparison of the skewness for these Jersey data with those of other breeds is given in table 3. These data show that butter-fat percentage of the four breeds, Jersey, Holstein-Friesian, Ayrshire and Guernsey, is plus and of small amount. The distribution of each breed has approximately the same numerical value for this constant.

Comparison of the skewness of the other milk solids with those of the butter-fat percentage distributions shows that these distributions are equal in their asymmetry within the limits of random sampling.

The comparison of the skewness of the percentage composition of the parts of the egg reveals the fact that the skewness of the percentage of shell is of about equal amount with those of the different parts of the milk. The skewness of the percentages of yolk and of albumin is slightly greater than that of any of the butter-fat percentages contained in the milk of the different breeds. In comparison with their probable errors the difference between these values is in certain cases undoubtedly significant in other cases the significance of the data is not so clear.

The conclusion appears justified that the frequency distributions for butter-fat percentage in cow's milk are slightly asymmetrical and that this skewness is in the plus direction.

ANALYTICAL CONSTANTS DESCRIBING THE VARIATION OF BUTTER-FAT PERCENTAGE AT DIFFERENT AGES

The general physical constants of butter-fat percentage having been considered, the further analysis of the variation for this butter-fat percentage requires a knowledge of the types of the frequency distribution which go to make up the lactation record of a cow during her life. No better illustration of the practical need for this information can be found than that given in a paper published on this subject by REITZ (1909). This author made a study of the inheritance of butter-fat production using the correlation method. Now butter fat is of course determined by two factors, the amount of milk and the butter-fat percentage. We have already shown that milk production is slightly skew in the plus direction. The data can be fitted, however, with a fair degree of accuracy, by a normal curve. Since the data used by REITZ deal with advanced-registry Holstein-Friesian records where a correction based on the assumption of normal curves must be made for the partial truncation of these curves by the requirements for entry, it follows that of equal importance with a knowledge of the distributions of milk production is a knowledge of the type of distributions of butter-fat percent. If these curves can also be fitted by normal curves without great error resulting, then and only then is the use of these corrected correlations justified.

The data on which this paper is based is excellent for the answer of such problems. The constants describing the distribution at the different ages in a cow's life are given in table 4.

The constants which have thus far not been considered include those requiring the higher moments in their derivation. Attention may first be directed to the values of the Betas. The values of β_1 range from 0.0433 ± .0414 to 0.3237 ± .1590. In no case do these values exceed three times their probable errors. The values of β_2 range from 2.6113 ± .2678 to 4.0557 ± 1.6557. The difference of β_2 from the value it should take for a normal curve is in no case more than two and one-half times its probable error. Out of the nine age groups the difference of β_2—3 is plus seven times and minus twice. The consistency of the sign rather than the amount of the difference leads to the conclusion that in general this constant for the distributions of butter-

TABLE 4

Analytical constants of variation in butter-fat percent at successive ages at test for Jersey milk (lactation period 8 months.)

Constants	2 yrs. 0 mo. to 3 yrs. 0 mo.	3 yrs. 0 mo. to 4 yrs. 0 mo.	4 yrs. 0 mo. to 5 yrs. 0 mo.	5 yrs. 0 mo. to 6 yrs. 0 mo.	6 yrs. 0 mo. to 7 yrs. 0 mo.
Mean	5.264 ± .018	5.278 ± .021	5.276 ± .020	5.235 ± .019	5.188 ± .022
Standard deviation	0.4662 ± .0129	0.4749 ± .0145	0.4415 ± .0139	0.4132 ± .0132	0.4445 ± .0153
Coefficient of variation	8.858 ± .347	8.998 ± .391	8.368 ± .374	7.894 ± .364	8.569 ± .419
μ_2	+ 9.6616	+ 10.0220	+ 8.6625	+ 7.5884	+ 8.7813
μ_3	+ 10.0850	+ 18.0499	+ 13.9935	+ 5.7195	+ 5.4141
μ_4	+ 335.8321	+ 359.7552	+ 250.9994	+ 205.6486	+ 243.9849
β_1	0.1128 ± .0949	0.3237 ± .1590	0.3012 ± .1343	0.0749 ± .0847	0.0433 ± .0414
β_2	3.5977 ± .5660	3.5818 ± .5718	3.3449 ± .4189	3.5713 ± .6102	3.1641 ± .3427
β_2-3	+ 0.5977	+ 0.5818	+ 0.3449 ± .4189	+ 0.5713	+ 0.1641
κ_1	+ 0.8571 ± .9573	+ 0.1925 ± .7911	— 0.2139 ± .5501	+ 0.9181 ±1.0643	+ 0.1983 ± .6956
κ_2	+ 0.1019 ± .1058	+ 1.3630 ±*	— 1.1350 ±6.1524	+ 0.0626 ± .0819	+ 0.1655 ±*
Skewness	+ 0.1333 ± .0490	+ 0.2635 ± .0556	+ 0.2744 ± .0583	———	———
Modal divergence	+ 0.0621 ± .0232	+ 0.0728 ± .0279	+ 0.1213 ± .0300	———	———
Mode	5.2014	5.2052	5.1547	———	———
Type of curve	IV	V	III	Normal	Normal
+ end of curve	∞	———	———	+ ∞	+ ∞
— end of curve	∞	1.8727	3.6672	— ∞	— ∞
y_0	17.8901	———	32.2018	32.1506	25.8484

* As the value of κ_2 is near the critical point of a number of type curves its probable errors have become very large.

TABLE 4 (continued)

Analytical constants of variation in butter-fat percent at successive ages at test for Jersey milk (lactation period 8 months.)

Constants	7 yrs. 0 mo. to 8 yrs. 0 mo.	8 yrs. 0 mo. to 9 yrs. 0 mo.	9 yrs. 0 mo. to 10 yrs. 0 mo.	10 yrs. 0 mo. and above	Total population
Mean	5.170 ± .022	5.155 ± .025	5.167 ±.035	5.134 ±.029	5.226 ± .007
Standard deviation	0.4322 ± .0158	0.4156 ± .0176	0.4818 ± .0249	0.4419 ± .0204	0.4492 ± .0052
Coefficient of variation	8.360 ± .456	8.062 ± .436	9.326 ± .688	8.607 ± .565	8.595 ± .100
μ_2	+ 8.3014	+ 7.6772	+ 10.3189	+ 8.6785	+ 8.9668
μ_3	+ 7.5805	− 5.0445	− 16.8024	− 6.3407	+ 6.8443
μ_4	+ 279.4886	+ 170.7971	+ 278.0460	+ 240.4522	+ 284.5584
β_1	0.1004 ± .1645	0.0562 ± .0521	0.2569 ± 1280	0.0615 ± .0756	0.0650 ± .0271
β_2	4.0557 ±1.6557	2.8978 ± .2801	2.6113 ± .2678	3.1926 ± .4917	3.5391 ± .2072
$\beta_2 - 3$	+ 1.0557	− 0.1022	− 0.3887	+ 0.1926	+ 0.5391
κ_1	+ 1.8100 ±3.0205	− 0.3731 ± .4890	− 1.5483 ± .3387	+ 0.2006 ± .8758	+ 0.8833 ± .3765
κ_2	+ 0.0434 ± .0686	− 0.1148 ± .2089	− 0.1350 ± .0776	+ 0.2335 ±*	+ 0.0563 ± .0279
Skewness	+ 0.1047 ± .0732	− 0.1357 ± .0718	− 0.5656 ± .2963	—	+ 0.1003 ± .0209
Modal divergence	+ 0.0453 ± .0317	− 0.0564 ± .0318	− 0.2725 ± .1446	—	+ 0.0572 ± .0091
Mode	5.1245	5.2117	5.4393	—	5.1687
Type of curve	IV	I	I	Normal	IV
+ end of curve	+ ∞	6.8043	6.0075	− ∞	+ ∞
− end of curve	− ∞	1.9480	3.4411	+ ∞	− ∞
y_0	21.4672	18.3530	10.0034	14.4901	154.207

* As the value of κ_2 is near the critical point of a number of type curves its probable errors have become very large.

fat percentage is a small plus quantity. The sign of κ_1 is plus six times and minus three times for the distributions at the different ages. In only one case is the numerical value of κ_1 greater than three times its probable error. The values of the probable errors are all quite large in comparison with the values of their probable errors for the criterion, κ_2, of the type of frequency curve.

In this respect these frequency distributions agree with those of the amount of milk produced by these same Jersey cows. All the constants of these two sets of data point to only a slight departure of these curves from the critical limit of more than one frequency type. This point is the place in the frequency surface where the use of the normal or Laplace-Gaussian curve is justified.

Before determining the type to be used for these data recourse must be had to the probable errors of the frequency distributions themselves. The laborious arithmetical work that must be done to determine these have been much simplified by the work of RHIND (1909). With the help of these tables, the type of the curves chosen to fit these distributions of frequency is thought to be such as to give the truest representation of the data.

These criteria call for the use of five different PEARSON (1905) frequency-type curves. The normal curve is required for three of the distributions; the type-four skew curve, unlimited in both directions, is required twice; type one, a curve limited in both directons is required twice; the transition types three and five limited in one direction only, are prescribed for one frequency each. The equations for these curves are given below.

Age at test 2 years to 3 years

$$y = 17.8901\left(1 + \frac{x^2}{3.9271}\right)^{-9.6974} e^{5.2895 \tan^{-1} \frac{x}{1.9817}}$$

Age at test 3 years to 4 years

$$\text{Log } y = 98.046145 - 54.4158 \log x - 516.7398\ 1/x$$

Age at test 4 years to 5 years

$$y = 32.2017 e^{-1.2381x} \left(1 + \frac{x}{9.9172}\right)^{12.2783}$$

Age at test 5 years to 6 years

$$y = 32.1506 e^{-x^2/15.1767}$$

Age at test 6 years to 7 years

$$y = 25.8484e^{-x^2/17.5625}$$

Age at test 7 years to 8 years

$$y = 21.4672\left(1 + \frac{x^2}{1.571736}\right)^{-5.8982} e^{2.0862 \tan^{-1} \frac{x}{1.2537}}$$

Age at test 8 years to 9 years

$$y = 18.3530\left(1 + \frac{x}{3.2638}\right)^{18.5614}\left(1 - \frac{x}{1.5925}\right)^{9.0570}$$

Age at test 9 years to 10 years

$$y = 10.0034\left(1 + \frac{x}{1.9982}\right)^{2.5291}\left(1 - \frac{x}{.5682}\right)^{.7191}$$

Age at 10 years and older

$$y = 14.4901e^{-x^2/17.3570}$$

Total population of Jersey cows.

$$y = 154.207\left(1 + \frac{x^2}{3.0094}\right)^{-9.4032} e^{4.1059 \tan^{-1} \frac{x}{1.7348}}$$

The histograms and the fitted curves for the frequency distributions of butter-fat percentage at the individual ages of the cow's life are shown in figure 1. All of the areas are reduced to a percentage base so that the ordinates and areas of each distribution are comparable with those of any other distribution directly.

The curves whose equations are given on page 264 graduate the data well as measured by the eye. The curve for the ninth year of age is quite a different shape from those of the other ages. The reason for this difference in shape is obscure. The lack of numbers in this distribution may possibly explain the difference in its shape.

The mathematical test of the accuracy of the graduation of these data is given by the values of P as calculated by PEARSON's formula (PEARSON 1905; ELDERTON 1901; VIGOR 1913).

Table 5 shows a considerable range in the value of P. Certain of the curves describe the observational data very accurately. Three of the graduations are mediocre. If we analyze the reason for this poor graduation further the following facts are brought out. Two curves gradu-

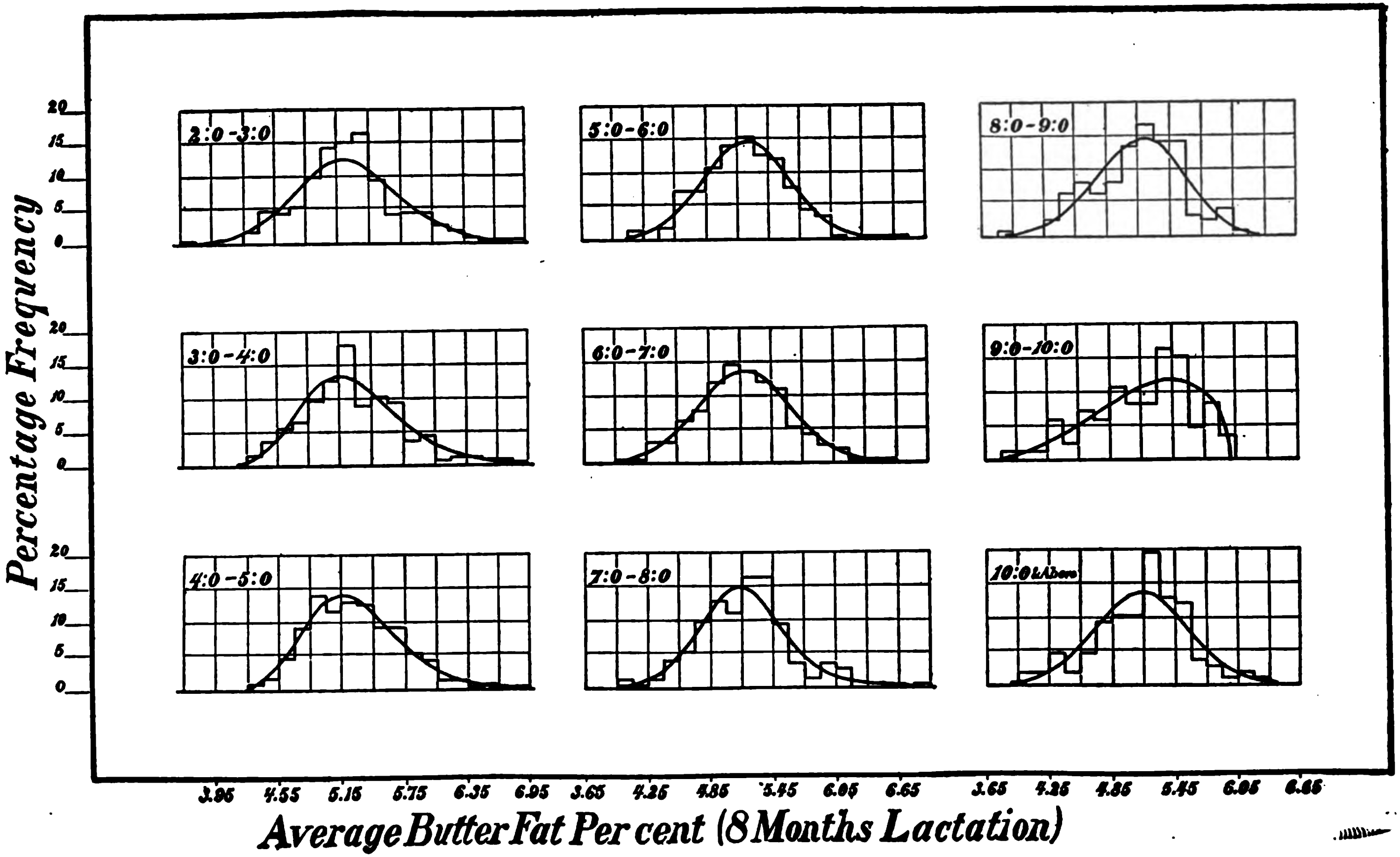

Figure 1.—Histograms and fitted curves showing the variation of the butter-fat percentage in Jersey milk for each year of age. The area of each curve is reduced to one so that distribution of a given age may be compared with any other at another age. The relative height of the modal ordinates is approximately the same throughout the cow's life. The irregularity of the original data is quite evident. The fitted curves strike through the observations well. The smoothed curves are all Pearson type curves the constants of which are seen in table 4.

TABLE 5

Values of P for the graduation of the theoretical curves to the observational data for butter-fat percentage of Jersey cattle.

Age at test	P
2 years to 3 years	0.3182
3 years to 4 years	0.4065
4 years to 5 years	0.5012
5 years to 6 years	0.9580
6 years to 7 years	0.9921
7 years to 8 years	0.4989
8 years to 9 years	0.3076
9 years to 10 years	0.7195
10 years and older	0.5008

ating the data for the first lactation at 2 years to 3 years have two ordinates which vary in opposite directions and contributed equally a total of 9.19 to the χ^2 value. If we neglect these two features the fit becomes 0.9036 or the rest of the graduation is most excellent.

The graduation of the three-year distribution is only fair because of one ordinate, the highest. This contributes 5.57 to the χ^2 value. Without considering this ordinate the value of P is 0.7439. Neglecting this one ordinate seems justified for if we examine figure 1 it is seen that this ordinate obviously goes higher than the general trend of the observations leads one to expect it to go.

The graduation for the eight-year-olds is not quite as good as that for the other curves. The observational data is quite uneven. The smooth curve fits the rough data better than it would seem from the value of P, as the contribution of one ordinate when subtracted from the next leaves the value of P 0.4747.

These facts make it seem that on the whole the graduation of the data by these type curves is better than would appear from the values of P taken by themselves.

The end of the expected range of any form of frequency data has much of general interest connected with it. Only two of these curves have this limit prescribed for both ends of the range. These curves are for the eight-year-olds and for the nine-year-olds. Two of the theoretical curves have the limited range at one end of the distribution. In both cases the end delineated is that for the low butter-fat percentage.

For the eight-year to nine-year age group the plus and minus ends of the range are at 6.8043 and 1.9480 percent, respectively. These limits are quite inclusive. As compared with the actual data the lower limit of the frequency curve is 1.85 percent lower than the actual data and the upper limit is .60 percent higher than the actual data. It is of interest to compare these Jerseys with a sample of the REGISTRY OF MERIT animals. In a limited sample of 1674 of these, gathered for other purposes, there were fifty whose butter-fat percentage had been determined on a year test between the ages, eight to nine. The end of the range for this set of data was 4.0 and 7.0 percent. These percentages are somewhat higher than would be expected for the first eight months on these cows. On the whole they fall in well with those of the pure-bred herd with which these data deal.

The nine-year-old group has the upper and lower limit of the theoretical curve prescribed at 6.01 and 3.44 percent. The upper limit is very close to the end of the actual data. The lower limit 3.44 is 1.5 percent larger than is the limit for the eight-years-of-age. The REGISTRY OF MERIT data similar to those mentioned for the eight-year-olds, show a range of 4.4 to 6.8 for the percentage in the year tests. The individual butter-fat percentages of these data are undoubtedly too high for the eight-months period of the same lactations. They do point to a difference in range of these REGISTRY OF MERIT animals from that of these data. The reason for this difference is in all probability to be found in the relatively few individuals contained in our data for the nine-year-old cows.

The lower limit of the range in the three-year-old group is quite similar to that of the eight-year-old group, 1.87 to 1.95. The lower limit of the four-year-old group agrees with that of the nine-year-old group. In either case these limits appear to be quite inclusive of the known data on Jersey cows.

All the curves (save the seven years of age) of butter-fat percentage at the different ages are of the mesokurtic type, as the values of β_2—3 are small and in each case are less than the probable error of β_2. The seven-year-olds have a kurtosis of over one in the plus direction. The value is, however, small in comparison with its probable error. The kurtosis is plus in eight of the nine age groups:

The variation curve and its constants for the total population of Jersey cattle is of considerable interest both for itself and in comparison with similar curves for other breeds of cattle. Table 4 gives the necessary constants for this calculation of the frequency curve suitable to fit the data. Figure 2 shows these data graphically.

The theoretical curve for these data is type IV as the values of β_1 and β_2 are such as to lead to a κ_2 of 0.0563. The curve is significantly

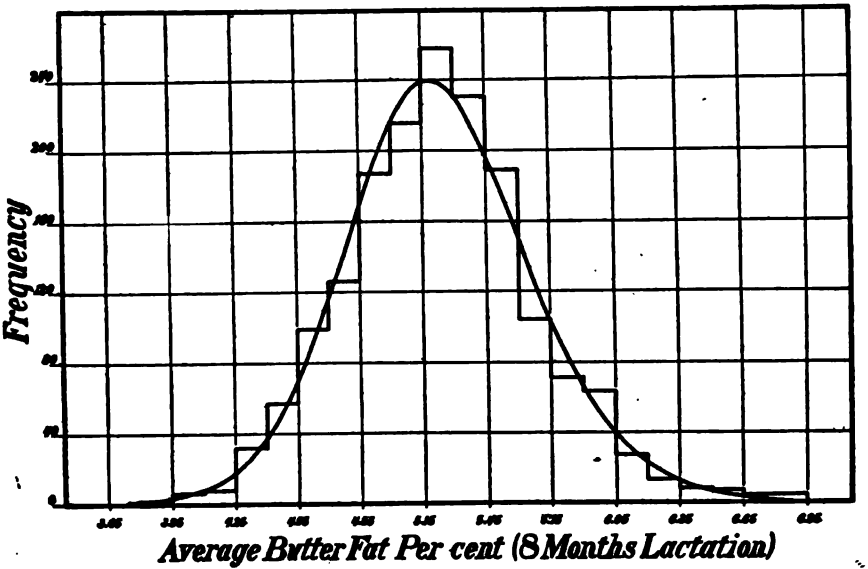

FIGURE 2.—Histogram and fitted curve showing variation of butter-fat percentage that may be expected to occur in a pure-bred Jersey without regard to age. It is evident that the curve strikes through the observations well. The slight positive skewness of the distribution is quite evident.

skew in the positive direction. The question arises whether this type of curve is typical for all breeds of milk-producing animals. VIGOR (1913) has furnished some information on the Ayrshires of Scotland which are comparable with these data save that the animals are from a large variety of farms. The constants for these data are $\beta_1 = 0.1104$, $\beta_2 = 3.2470$ leading to a κ_2 of 0.5231. This curve is consequently of type IV, also. Other data of somewhat less value, as they have been subject to some selective influences have been tabled for Holstein-Friesian cows by the author (GOWEN 1920). The data on Holstein-Friesian cows[2] lead to values of $\beta_1 = 0.1432$ $\beta_2 = 3.6752$ and $\kappa_2 = 0.1214$. These data call for the same type of curve as that for this pure-bred Jersey herd. It would seem from constants for butter-fat percentage that these curves belong to the type IV group. Unfortunately this conclusion does not hold for some unpublished data on the pure-bred advanced-registry Guernsey cows. The constants $\beta_1 = 0.0581$, $\beta_2 = 3.0468$

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[2] It should be mentioned that the distributions of either the Holstein-Friesian butter-fat percentage do not show any evidence of truncation due to the butter-fat requirement for entry of these curves into their respective herd books. It is probable therefore, that these records are but little influenced by this requirement.

and $\kappa_2 = -0.5472$ leading to the type I curve. These data are, of course, subject to the same limitations as are the data on the Holstein-Friesians. These values of the Betas are such as to lead to a value of κ_1 which is not significant in comparison with its probable error. Also the value of the probable error of κ_2 is very large, consequently it is likely that other types of curves than the type I indicated by the criterion would fit the data well. The case of the distribution of Guernsey butter-fat percentage must, however, be considered against the conclusion that the distributions of butter-fat percentage in cow's milk irrespective of age and breed all belong to the type IV group of curves.

The frequency curve for the percentage of the solids-not-fat contained in the Holstein-Friesian milk is of interest as it is on the variation of the albumin and sugar concentration of the milk. The constants for this distribution are $\beta_1 = 0.2114$, $\beta_2 = 3.9872$ and $\kappa_2 = 0.1257$ leading to a type IV curve. The variation of the concentration of the other solids than the butter-fat percentage of cow's milk is consequently entirely similar in type with that of the butter-fat percentage of Holstein-Friesian milk.

With these facts in mind the justification for treating the distributions of butter-fat percentage as normal may be considered. The distributions have been shown to be slightly skew. The skewness is not enough to make a large error if the distributions were considered normal. The constants for the distribution deviate only slightly from those required for the normal curve. These facts make it likely that no grave error would creep into the determination of parent-and-offspring correlation by the method for doubly truncated data which has been used by REITZ (1909).

THE CORRELATION OF JERSEY EIGHT-MONTHS BUTTER-FAT PERCENTAGE WITH AGE OF THE COW AT COMMENCEMENT OF TEST

Increase in age of the cow from the time she is a heifer to the time she is at her maximum productivity at 7 years has been shown to bring about a logarithmic increase in the quantity of eight-months milk produced by that cow. The effect of increasing the age of the cow on the quality is of equal interest to the student of the physiological behavior of the mammary gland. The data for this comparison in the herd of pure-bred Jersey cows with which this study deals are shown in table 6.

GOWEN, J. W., V T PERCENTAGE WITH AGE IN JERSEY CATTLE

LE 6

Correlation surfa test for Jersey cattle. (Lactation period 8 months.)

Age at test	percentage													
	5.00-5.15	5.15-5.30	5.30-5.45	5.45-5.60	5.60-5.75	5.75-5.90	5.90-6.05	6.05-6.20	6.20-6.35	6.35-6.50	6.50-6.65	6.65-6.80	6.80-6.95	Total
1.6- 2.0	3	3	7	7	3	4	1	1						38
2.0- 2.6	35	37	42	25	10	13	10	5	5	2			1	250
2.6- 3.0	7	7	6	3	2		3	3			1	1		46
3.0- 3.6	18	34	15	19	16	6	8	1	1	1		1	1	158
3.6- 4.0	13	10	7	6	7	3	3	1	2	2	2	1		87
4.0- 4.6	15	15	18	13	13	9	4	3	2	1			1	132
4.6- 5.0	12	15	11	8	8	3	6		1		2			98
5.0- 5.6	18	13	14	14	10	6	3					1		107
5.6- 6.0	13	21	14	13	7	4	5	1		1	1			115
6.0- 6.6	10	13	9	13	2	5	3	1	1	1	1			90
6.6- 7.0	18	13	14	8	8	3	2	3						102
7.0- 7.6	6	12	16	5	5	2	3	2					1	81
7.6- 8.0	13	16	12	11	1		3	3						90
8.0- 8.6	11	10	10	10	3	1	4							70
8.6- 9.0	6	11	8	8	1	2	1	1						57
9.0- 9.6	3	4	11	5	2	6	2							48
9.6-10.0	4	3	3	8	2	1	1							37
10.0-10.6	2	4	3	6	1	1			1					29
10.6-11.0	1	3	4	2		1	1							21
11.0-11.6	3	4	5	1	1	1		1						18
11.6-12.0		6		1	1									12
12.0-12.6	1	1			1			1						8
12.6-13.0	2		1	1										8
13.0-13.6	1	2												6
13.6-14.0			1	1										2
14.0-14.6	1	1												2
14.6-15.0				1										1
Total	6	258	231	189	104	71	63	27	13	8	7	4	4	1713

The correlation and its accompanying constants for these two variables are as follows:

$r = -0.1126 \pm .0161$; $\eta = 0.1478 \pm .0159$; $\eta^2 - r^2 = 0.0092 \pm .0031$

From these constants it is clear that there is a slight significant relation between the age of the cow and the concentration of the butter fat contained in her milk. The value of η corresponds well with that of r except that it is different in sign as η by its derivation is a positive quantity. The regression is clearly a linear one as the values of the constants to measure the linearity ($\eta^2 - r^2$) are less than three times their probable error ($0.0092 \pm .0031$).

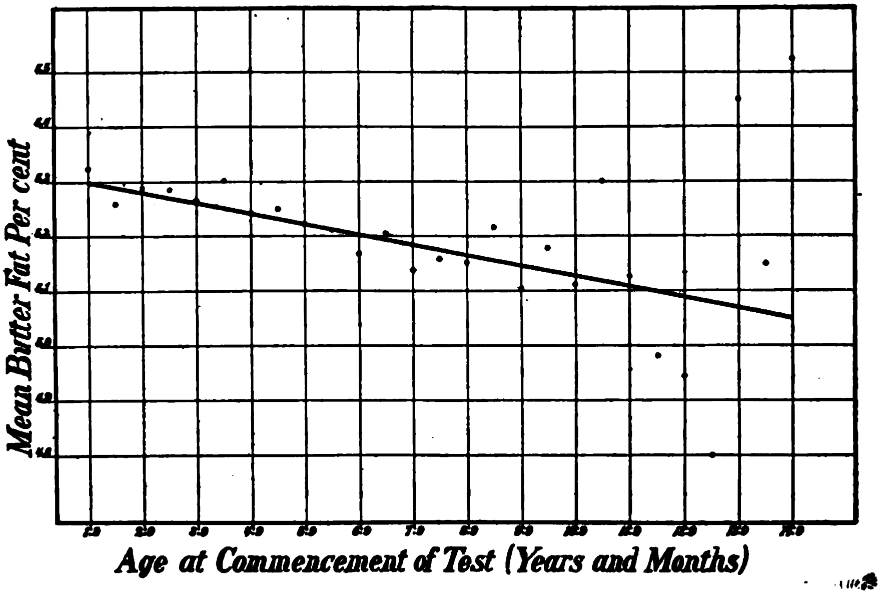

FIGURE 3.—Diagram showing the observed means and theoretical means for Jersey butter-fat percentage of eight-months milk at any given age. The observed means are calculated from table 6. The curve as a whole describes the observations well. The actual means are represented by the small circles.

Figure 3 shows the means of the butter-fat percentage for each successive array of age calculated from table 6. The equation to these means is easily determined from a knowledge of the correlation and the standard deviation of the two variables. When the suitable calculations are made the equation is shown to be:

Butter-fat percentage = 5.3320 — .0191 × age at test.

A glance at the figure shows that the fit of this line is very good. The value of P, calculated by the method of SLUTSKY (1914) except that the calculated standard deviations are used in place of the observational, is 0.8715 or the fit is very good.

These facts lead to the following law governing the action of the mammary gland in these Jersey cows: As the age of a given cow advances the relative capacity of this cow to secrete butter-fat decreases by a small but significant amount.

The curve for the variation (standard deviation) of these data at each age is shown in figure 4. The small number of observations in the older ages makes this curve quite irregular. Its general trend seems to be that of a line with a slight downward slope. The observation at twelve years and three months of age is clearly abnormal. This is undoubtedly due to lack of numbers in this class and also in the other older class. Before attempting to describe these data by any theoretical function it seemed best in view of these facts to use the first smooth of the observational data from the age of eleven years and three months. The observations by this point were considered too scanty to warrant using them in the calculations. The linear nature of the curve calls for a linear function. The line determined from the data by the least-square method is shown in figure 4.

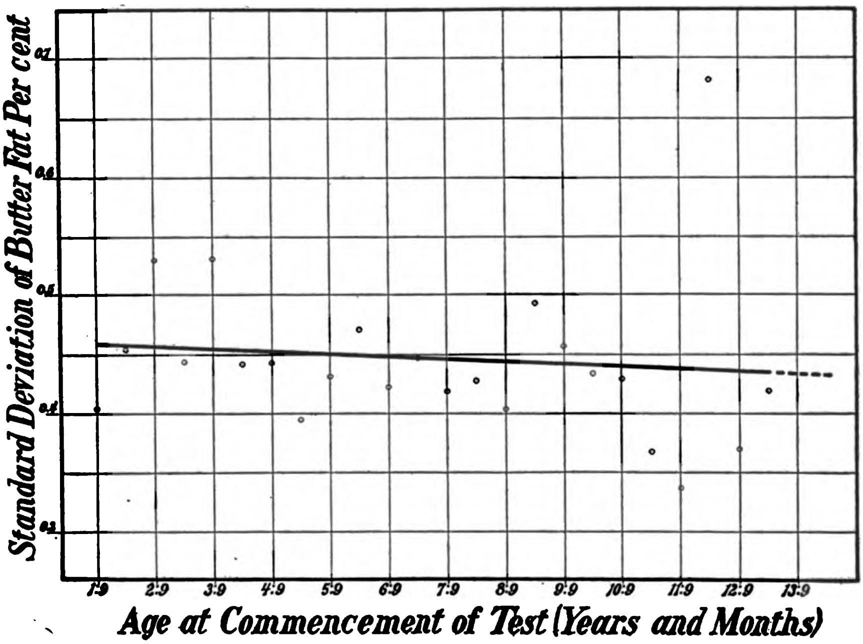

FIGURE 4.—Observational and theoretical curve for the standard deviations of butter-fat percentage for the successive ages at which the lactation commenced. The theoretical curve is a linear function. The data on which this curve is based are seen in table 6.

The equation of this line is

Standard deviation of butter fat of given age = $0.4567 - .0009 \times$ age given.

These curves clearly show a very slight downward trend of the standard deviations with increasing age of the cow. Since the means also decreases and as a decrease would be expected in the standard deviation if the means decrease, it is doubtful if any great significance can be attached to this decrease in the standard deviations.[1]

In comparison with the previous curves on the milk production for the same cows plotted on the same age basis (GOWEN 1920) the curve for the butter-fat percentage shows that while the milk production rises logarithmically to a maximum and then falls off more slowly, the butter-fat percentage actually is slightly decreasing in this milk, as the age increases.[2] This means that while the mass of butter fat produced by a cow follows in general the same kind of function as does the milk, there is this difference, viz., the butter fat relative to the milk is always decreasing slightly in amount.

This fact of a slight negative correlation and a consequent decline in the mean butter-fat percentage produced with the advancing age of the Jersey cow is interesting in comparison with the known facts for other breeds. As previously shown by the writer (GOWEN 1919) the correlation between age and butter-fat percentage for the year-test Holstein-Friesian cows is $-0.0546 \pm .0181$. VIGOR has shown the correlation between these same variables to be $-0.2744 \pm .0255$ for Ayrshire cattle (the author has checked the result and found it correct). From unpublished data of the writer the correlation of advanced-registry Guernsey cattle for these same variables is $-0.1174 \pm .0134$. The correlation for the Jersey is equal to $-0.1126 \pm .0161$. Two of these correlations are based on advanced-registry data and may be considered as subject to a selective influence on the data. The distributions do not look as if such a disturbing factor had been present as there is no evidence of truncation and as shown in a previous part of the paper the frequency constants agree quite well with those of the Jerseys and Ayrshires known to be untruncated. It seems therefore that the constants above should be directly comparable as to the relation of age and butter-fat percentage of these breeds.

The Jersey correlation coefficients do agree very closely with those of the Guernseys. The Ayrshires do not agree at all with any of the other breeds for difference of the correlations of Ayrshire and Jersey is 0.1618 ± .0301 or 5.4 times its probable error. The multiple times the probable error is greater for the Holstein-Friesians. The Holstein-Friesians correlation coefficient compared with that of the Jerseys is probably not significantly different as it is only 2.4 times the probable error. The correlation for the Holstein-Friesian age and percentage of butter fat produced is probably not significant.

The Ayrshire results are obtained under the conditions of Scotland, whereas the other results are on cattle kept in this country. This may possibly account for the difference in influence of age on butter-fat concentration of Ayrshire cows as compared with these other breeds or it may equally well mean that the Ayrshires are innately different from the other breeds.

The correlation for the Holstein-Friesian in comparison with the correlation for the other breeds is small. It does show the same sign as the other correlations.

These considerations taken together lead to the conclusion which may be expressed tentatively as follows: Each increment of time added to a cow's life causes a slight decline in the concentration of butter fat that the cow's mammary gland can secrete into the milk.

THE CORRELATION BETWEEN THE BUTTER-FAT PERCENTAGE OF ONE LACTATION AND THAT OF SUCCEEDING LACTATIONS

Up to this point the discussion has been entirely directed toward the analysis of the influence of age on the percentage of butter fat produced in a given lactation and the variability of this butter-fat percentage. In the present section the phase of the problem dealing with butter-fat percentage of one lactation in relation to that of another lactation will be considered.

Little or no analysis based on concrete data has been made on this problem, yet obviously on a knowledge of these relations depends the justification for many of the practices now extant in dairy husbandry as well as laying the foundation for the solution of many problems connected with the secretion-of-butter-fat percentage itself. The existing information concerning butter-fat secretion is largely empirical. It is commonly said that the great butter-fat-producing machines of to-day are due to the use of such methods by these cattle breeders. In the widest sense this is no doubt true although such a mode of procedure tells us nothing about the biological factors underlying the advance in butter-fat percentage, or the laws by which it is governed. In such cases chance and luck play a very important part in the improvement.

It is in the removal of these disturbing factors and making the improvement less haphazard that exact numerical analyses find their place. The solution of the problems connected with butter-fat production are complex and need to be approached from many angles. This section of the present investigation was undertaken in the hope that some knowledge of the intra-individual variation with regard to the relative concentration of this butter fat from lactation to lactation would throw some definite light on these problems. The homogeneous nature of the material is especially favorable to this problem.

Naturally the problem resolves itself into a study of the relative strength and precision of action of the inherited complex possessed by the cow working in conjunction with and in opposition to the environmental changes. If heredity plays a large part in the production of a cow the position of the cow in the frequency curves discussed in the earlier part of the paper will remain approximately the same from lactation to lactation; if on the other hand inheritance of butter-fat production is weak in comparison with the influence of the shifts in environment, the position of the cow in our frequency curves will change materially from lactation to lactation. The preliminary steps in this analysis should include a study of each population for each of the compared distributions to be sure that the samples are the same as those of the whole population for butter-fat percentage of all cows of that age.

The reason for this is made quite clear by considering an example. Suppose a population of Jersey cows is subject to selection on the basis of their three-year-old records. Should a correlation coefficient be determined between the butter-fat percentage of the lactations at three years old and the lactation at four years old the true correlation coefficient would not be obtained. Since the three-year-olds were kept to four on the basis of their third-year lactation being high, there must have been a portion of these three-year-olds records curtailed from the general distribution of butter-fat percentage of Jersey cows because of their low yield. The same reasoning holds for the four-year-old distribution. Such a correlation table could be doubly curtailed and the true correlation coefficient could only be determined by special methods (REITZ 1909). It becomes necessary therefore to show that no evident curtailment of the data has taken place before any reasoning from the correlation coefficient determined on such data can be made. The data for this comparison are taken from the correlation tables beginning with table 7 and ending with table 41.

The influence of the length of time the cow is retained in the herd on the mean butter-fat percentage of a given age

TABLE 7

Correlation surface for percentage of butter fat in the eight-months milk production at two years old and percentage of butter fat in the eight-months milk production at three years old for pure-bred Jersey cows.

3 years old	2 years old: 3.80-4.00	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	Total
4.20-4.40			1		2	1									4
4.40-4.60	1		1	3	2	1							1		9
4.60-4.80				3	4	6	5	3							21
4.80-5.00				1	2	5	6	2							16
5.00-5.20			1	2	1	3	8	7	3	1		1			27
5.20-5.40				1	3	1	4	9	8	1		1	1		29
5.40-5.60				1		1	2	4	8	2	1	2	1		22
5.60-5.80				2		1	4	5	8	2	5	1			28
5.80-6.00						1		1	2	3			1		8
6.00-6.20									1		1				2
6.20-6.40					1							1	1		3
6.40-6.60					1		1					1		1	4
6.60-6.80							1			1					2
Total	1	0	3	13	16	20	31	31	30	10	7	7	5	1	175

The tabled constants are presented in the following order, (1) the mean butter-fat percentage for each of the compared populations (table 9); (2) the standard deviation of each of the compared populations (table 17),—and the coefficient of variation of each of the compared populations (table 20). The vertical columns of each table present the means, standard deviations, etc., of the age indicated when correlated with each of the lactation records made at the age on the left of the table. Thus, the means of the butter-fat percentage of the two-year-old group that is correlated with the three-year-old group, is 5.228 ± .024 percent of butter-fat in the eight-months milk. The values of this mean butter-fat percentage in this column should be the same within the limits of random sampling if the population of milk production of the two-year-olds is the same for the cows kept to their three-year-old lactation, as it is for the cows kept to their seven- or eight- or nine-year-old lactations. These remarks, which are directed for illustrative purposes to the two-year-old group only, are equally applicable to any other group as one progresses from the left to the right of the table.

TABLE 8

Correlation surface for percentage of butter fat in the eight-months milk production at two years old and percentage of butter fat in the eight-months milk production at four years old for pure-bred Jersey cows.

4 years old \ 2 years old	4.40-4.60	4.20-4.40	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.20-4.40		1	1									2
4.40-4.60	1	1	2									4
4.60-4.80		2	3	3	1	1	2		1			13
4.80-5.00	1	3	2	4	10	4	3					27
5.00-5.20			4	4	4	2	3	1				18
5.20-5.40		2		3	6	7	8		1			27
5.40 5.60		1		5	1	3	10	2	1	1		24
5.60-5.80		1	1		2	4	1	5	3	2		19
5.80-6.00			1			1		1	1	2	2	8
6.00-6.20					1	2	1				1	5
6.20-6.40					1		1	1	1			4
6.40-6.60				1				1		1		3
6.60-6.80												0
6.80-7.00							1					1
Total	2	11	14	20	26	24	30	11	8	6	3	155

Table 9 presents the data for the comparisons of these mean butter-fat percentages for the different age groups. Figure 5 shows graphically the means for each age group seen in table 9.

TABLE 9

Mean butter-fat percent of a given year when correlated with other given years.

Ages with which correlated	Age correlated			
	2.0-3.0	3.0-4.0	4.0-5.0	5.0-6.0
2.0- 3.0	————	5.283 ± .025	5.319 ± .026	5.281 ± .026
3.0- 4.0	5.228 ± .024	————	5.270 ± .026	5.258 ± .025
4.0- 5.0	5.239 ± .024	5.261 ± .027	————	5.257 ± .025
5.0- 6.0	5.242 ± .028	5.276 ± .028	5.284 ± .026	————
6.0- 7.0	5.247 ± .029	5.219 ± .028	5.222 ± .027	5.243 ± .026
7.0- 8.0	5.248 ± .034	5.173 ± .039	5.193 ± .027	5.229 ± .025
8.0-10.0	5.279 ± .032	5.253 ± .029	5.156 ± .026	5.212 ± .023
10.0 and above	5.294 ± .051	5.184 ± .053	5.144 ± .039	5.164 ± .035

TABLE 9 (continued)

Mean butter-fat percent of a given year when correlated with other given years.

Ages with which correlated	Age correlated			
	6.0-7.0	7.0-8.0	8.0-9.0	10.0 and above
2.0- 3.0	5.228 ± .026	5.140 ± .029	5.163 ± .028	5.179 ± .049
3.0- 4.0	5.193 ± .026	5.147 ± .028	5.183 ± .025	5.174 ± .050
4.0- 5.0	5.155 ± .027	5.161 ± .028	5.156 ± .027	5.152 ± .042
5.0- 6.0	5.178 ± .025	5.147 ± .024	5.168 ± .023	5.096 ± .040
6.0- 7.0	———	5.103 ± .024	5.167 ± .022	5.144 ± .038
7.0- 8.0	5.190 ± .027	———	5.150 ± .022	5.175 ± .036
8.0-10.0	5.183 ± .023	5.147 ± .023	———	5.155 ± .026
10.0 and above	5.205 ± .043	5.153 ± .034	5.165 ± .029	———

The dashed spaces in this table are due to the fact that no correlations could be determined for the given year with itself. Similarly the dotted lines in figure 5 were used to connect means of the age immediately preceding with the means of the age immediately following the given year.

The variation of the highest and lowest mean butter-fat percentage for the individuals of the eight-year group when these cows also have a lactation at another age is less than that of any other group. The greatest difference in the highest and lowest butter-fat percentage is for the four-year-old to five-year-old group; the swing of these means has its highest point for those cows which have lactations at two years and its lowest point for those animals which have other lactations at 10 years, the difference being 0.175 percent of butter fat. This difference as compared with the probable errors of their means is slightly more than three times the probable error. While such a difference is significant mathematically, it is doubtful if any great weight should be attached to it as the numbers in the four-year group with lactations at two years old are not very large.

The graphical presentation of these means is seen in figure 5. In this figure the mean butter-fat percentage is given at the left. The line at the top of the figure represents the mean butter-fat percentage of the two-year-old cow which also had lactation records at the other ages shown at the lower margin of the figure. Thus the point where the 3.0-to-4.0 vertical crosses this line represents the mean butter-fat percentage of the two-year-old cow's lactation where these same cows were also tested at three years old. The point where the 4.0-to-5.0 vertical crosses this line represents the mean of the two-year-old cow's lactation where these same cows were also tested at four years old.

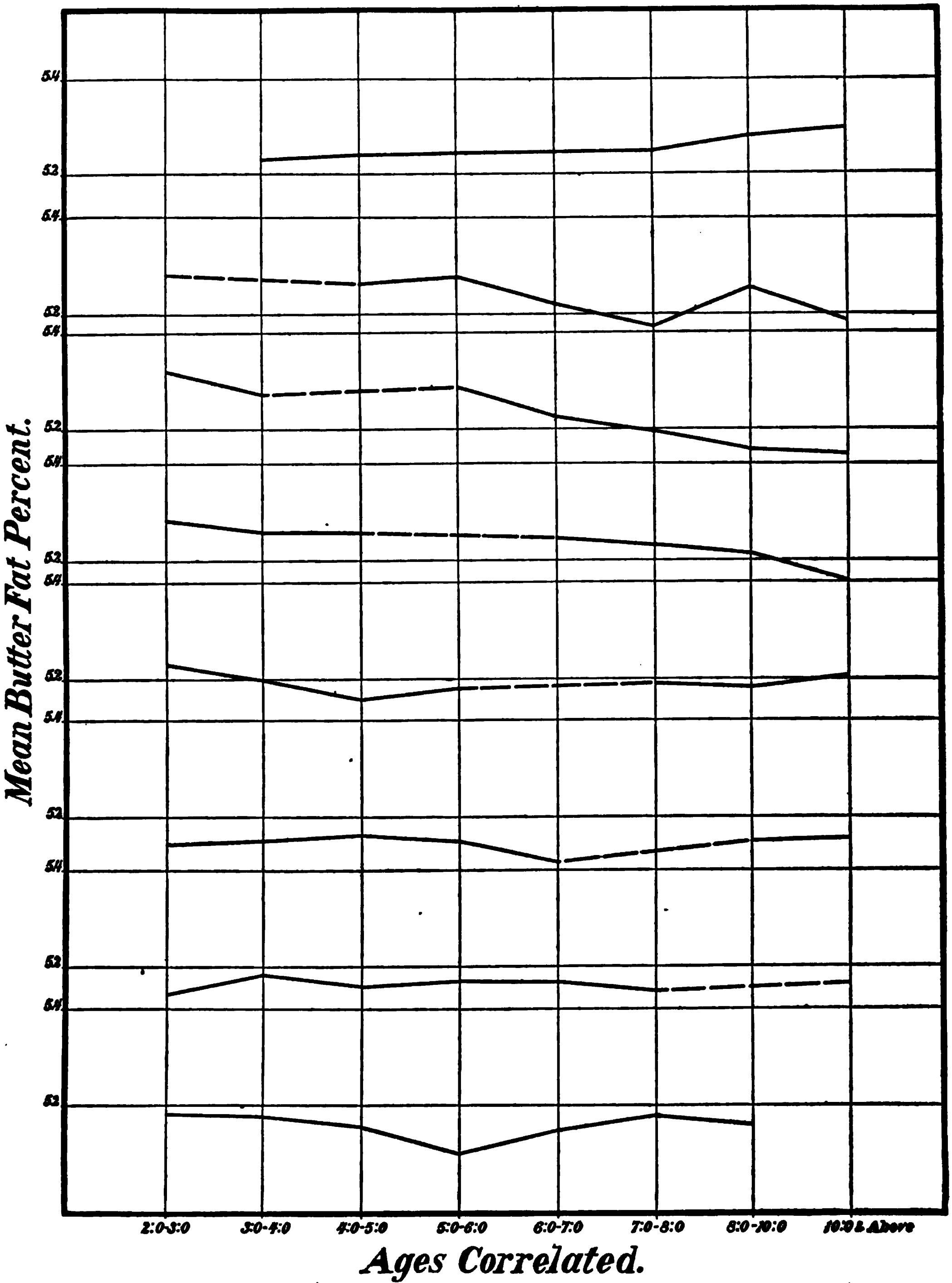

FIGURE 5.—Curves showing the mean butter-fat percentage contained in the eight-months milk of a cow at a given age for each successive (lactation) age with which the given (lactation) age is correlated. The ordinates are percentages of butter fat. The abscissae are the ages of the groups with which the given (lactation) ages are correlated. Proceeding from top to bottom of the figure the first line represents the mean butter-fat percentage of the 2-year-old to 3-year-old group of cows; the second the 3-year-old to 4-year-old group; the third the 4-year-old to 5-year-old group; the fourth the 5-year-old to 6-year-old; the fifth the 6-year-old to 7-year-old; the sixth the 7-year-old to 8-year-old; the seventh the 8-year-old to 10-year-old, and last, the 10-year-and-older group of cows. The lines are on the whole quite regular and in general do not diverge greatly from a line parallel with the base of figure.

TABLE 10

Correlation surface for percentage of butter fat in the eight-months milk production at two years old and percentage of butter fat in the eight-months milk production at five years old for pure-bred Jersey cows.

2 years old

5 years old	3.80-4.00	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20						1								1
4.20-4.40														0
4.40-4.60			2	1	2	1								6
4.60-4.80				4	2	2	3	1						12
4.80-5.00	1		1	2	1	4	3	2	2		1			17
5.00-5.20					2	3	4	8	6	2				25
5.20-5.40				4	1	3	1	5	2	2	1	1	1	21
5.40-5.60					1	2	5	4	9		2		1	24
5.60-5.80					2		2	3	2	1	1	1		12
5.80-6.00								1	2	2	2	2	2	11
6.00-6.20								1			1		1	3
6.20-6.40												1		1
6.40-6.60														0
6.60-6.80									1		1			2
Total	1	0	3	11	11	16	18	25	24	7	9	5	5	135

TABLE 11

Correlation surface for percentage of butter fat in the eight-months milk production at two years old and percentage of butter fat in the eight-months milk production at six years old for pure-bred Jersey cows.

2 years old

6 years old	3.80-4.00	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20					1									1
4.20-4.40				1	1		1							3
4.40-4.60				2	1	1	1							5
4.60-4.80				1	2	2	1		1					7
4.80-5.00	1			2		4	2	3	2					14
5.00-5.20			1		1	5	4	7	2	1	1			22
5.20-5.40			1		1	3	2	4	8	2	2			23
5.40-5.60				1	1	1	2	6	3	2	2	2		20
5.60-5.80								2	8				1	11
5.80-6.00					1							2	1	4
6.00-6.20									1		2			3
6.20-6.40													1	1
Total	1	0	2	7	9	16	13	22	25	5	7	4	3	114

The next line down in the figure represents the three-year-old cows' mean butter-fat percentage. The points on this curve represent the three-year-old means of those cows which were also tested at the ages indicated at the bottom margin of the figure. The third line, fourth line, etc., represent the four-year-olds' mean butter-fat percentage, the five-year-olds' mean butter-fat percentage, etc.

The lines generated by the mean butter-fat percentages of each of these age groups should, of course, be parallel with the base of the figure within the limits of random sampling if the populations of the eight-months butter-fat percentages for the given age are the same irrespective of the age at which the subsequent tests were made.

The curve for the mean butter-fat percentage of the two-year-old group (the top curve of figure 5) is as close to a straight line parallel with the base of the figure as could be expected. At no place is the divergence from such a line more than 0.033 percent of butter fat or there is not as much divergence as three times the probable error of the determination. Likewise the slight rise in the line does not equal more than the probable error of the difference in the height of the two ends of the line. It is clear that for these two-year-olds no differential sampling from the two-year population was practiced in determining what cows were to be kept for subsequent lactations.

TABLE 12

Correlation surface for percentage of butter fat in the eight-months milk production at two years old and percentage of butter fat in the eight-months milk production at seven years old for pure-bred Jersey cows.

	2 years old													
7 years old	3.80-4.00	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20					2									2
4.20-4.40			1	1	1									3
4.40-4.60	1			1	1	1								4
4.60-4.80			1	1	1	1	2	1						7
4.80-5.00						2	3		4	1	1			11
5.00-5.20				2	1	2	1	5	8					19
5.20-5.40				1		4	5	5	4	1	2	1		23
5.40-5.60					1			4	2	1	1	3	1	13
5.60-5.80							1							1
5.80-6.00								1	1		2	1		5
6.00-6.20											1			1
Total	1	0	2	6	7	10	12	16	19	3	7	5	1	89

TABLE 13

Correlation surface for percentage of butter fat in the eight-months milk production at two years old and percentage of butter fat in the eight-months milk production at eight and nine years old for pure-bred Jersey cows.

2 years old

8 and 9 years old	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	Total
4.20-4.40			1			1	1				3
4.40-4.60	1	4	4	1		1					11
4.60-4.80			3		3		1		1		8
4.80-5.00	1				2		2	1			6
5.00-5.20				2	5	5	2	1			15
5.20-5.40	1	1		3	2	4	4	2	2	1	20
5.40-5.60				3	1	7	5	1	3	2	22
5.60-5.80						1	1		3	2	7
5.80-6.00						1			1	1	3
Total	3	5	8	9	13	20	16	5	10	6	95

The curve for the three-year-old lactation records, while more variable than the two-year-old groups, leads to the same general conclusion as that expressed for the two-year-old group. All of the other age curves likewise show no difference in the individuals within that group which are kept for subsequent lactation records. Summing up the evidence, it appears clear that no selection of animals to be kept as milk-producers in subsequent lactations on the basis of these butter-fat percentages contained in their milk is evidenced by the mean butter-fat percentage of any age in comparison with the other lactation at which the cow was known to be milked.

The standard deviations of the butter-fat percentage as influenced by the length of time the cow is retained in the herd

A similar analysis for the effect of selection of cows at a given age on the basis of their butter-fat percentage to be retained in the herd as milkers in after years is given in table 17. The arrangement of the data is entirely similar to that of table 9. The vertical columns give the standard deviations (without SHEPPARD'S correction) for the age of the cow indicated. The constants from top to bottom of a vertical column are those of the given year when the cow was also kept in the herd and tested at the ages indicated on the left margin of the table. The data for the calculation of these standard deviations are taken from correlation tables 7 to 41.

TABLE 14

Correlation surface for percentage of butter fat in the eight-months milk production at two years old and percentage of butter fat in the eight-months milk production at ten-and-above years old for pure-bred Jersey cows.

10-and-above years old	2 years old										
	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	Total
4.00-4.20		1									1
4.20-4.40		1									1
4.40-4.60	1										1
4.60-4.80				2		1					3
4.80-5.00				1			1		1		3
5.00-5.20			1	1			4		1		7
5.20-5.40				1	1	3	1				6
5.40-5.60					1	1	3		2	1	8
5.60-5.80											0
5.80-6.00					1	1			1		3
Total	1	2	1	5	3	6	9	0	5	1	33

TABLE 15

Correlation surface for percentage of butter fat in the eight-months milk production at three years old and percentage of butter fat in the eight-months milk production at four years old for pure-bred Jersey cows.

4 years old	3 years old														
	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	6.60-6.80	6.80-7.00	Total
4.20-4.40	1		1												2
4.40-4.60	1		3												4
4.60-4.80	1	2	3		4	4	1	1							16
4.80-5.00		2	4	7	8	3	2	2							28
5.00-5.20	1	2	3	2	3	2	3	1	1						18
5.20-5.40			1	7	5	7	4	2	1						27
5.40-5.60			1	1	3		10	3		1					19
5.60-5.80			2			4	4	3	2		1		1		17
5.80-6.00						1	4	1			2			1	9
6.00-6.20		1						1		1		1			4
6.20-6.40									1	1					2
6.40-6.60									1			1			2
6.60-6.80															0
6.80-7.00															1
Total	4	7	18	17	23	21	28	15	6	3	3	2	1	1	149

TABLE 16

Correlation surface for percentage of butter fat in the eight-months milk production at three years old and percentage of butter fat in the eight-months milk production at five years old for pure-bred Jersey cows.

5 years old \ 3 years old	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	Total
4.00-4.20			1										1
4.20-4.40													0
4.40-4.60	2	3		1									6
4.60-4.80		3	5	3	2	1		1					15
4.80-5.00		1	2	3	2	2	2	1					13
5.00-5.20			1	3	6	3	2	4	1	3			23
5.20-5.40	2	1	2	3	4	4	6	2	1				25
5.40-5.60					4	10	7	4	2				27
5.60-5.80					1	2	3	3	1			1	11
5.80-6.00				1		3			1	1	3		9
6.00-6.20							1					1	2
6.20-6.40								1					1
6.40-6.60													0
6.60-6.80								1					1
Total	4	8	11	14	19	25	21	17	6	4	3	2	134

The graphical presentation of these data is seen in figure 6, the values of the standard deviation being represented as ordinates and the ages at which the cows had another lactation as the abscissae. The arrangement of the curves is similar to that for the mean butter-fat percentage

TABLE 17

Standard deviation for butter-fat percentage of a given year when correlated with other given years.

Ages with which correlated	Age correlated 2.0-3.0	3.0-4.0	4.0-5.0	5.0-6.0
2.0- 3.0	——	0.496 ± .018	0.476 ± .018	0.447 ± .018
3.0- 4.0	0.471 ± .017	——	0.464 ± .018	0.421 ± .017
4.0- 5.0	0.443 ± .017	0.494 ± .019	——	0.442 ± .018
5.0- 6.0	0.489 ± .020	0.474 ± .020	0.452 ± .018	——
6.0- 7.0	0.462 ± .021	0.427 ± .020	0.429 ± .019	0.440 ± .019
7.0- 8.0	0.475 ± .024	0.566 ± .027	0.409 ± .019	0.400 ± .018
8.0-10.0	0.457 ± .022	0.443 ± .021	0.410 ± .018	0.403 ± .016
10.0 and above	0.439 ± .036	0.345 ± .038	0.425 ± .028	0.391 ± .025

TABLE 17 (continued)

Standard deviation for butter-fat percentage of a given year when correlated with other given years.

Ages with which correlated	Age correlated			
	6.0-7.0	7.0-8.0	8.0-10.0	10.0 and above
2.0- 3.0	0.415 ± .019	0.401 ± .020	0.408 ± .020	0.421 ± .035
3.0- 4.0	0.403 ± .019	0.405 ± .020	0.388 ± .018	0.325 ± .036
4.0- 5.0	0.428 ± .019	0.430 ± .020	0.428 ± .019	0.452 ± .029
5.0- 6.0	0.416 ± .018	0.388 ± .017	0.408 ± .016	0.438 ± .028
6.0- 7.0	————	0.396 ± .017	0.404 ± .016	0.433 ± .027
7.0- 8.0	0.437 ± .019	————	0.410 ± .016	0.455 ± .026
8.0-10.0	0.422 ± .017	0.424 ± .016	————	0.430 ± .018
10.0 and above	0.486 ± .030	0.433 ± .024	0.484 ± .021	————

curves (figure 5). The curve for the two-year-olds is at the top of the figure and the curve for the ten-year-and-older group is at the bottom of the figure. The lines should, of course, be parallel with the base of the figure if there has been no selection practiced. If selection has taken place at any given age of the cow's life, it would be expected to curtail the low-butter-fat-test cows and thus reduce the standard deviation of the whole population kept for subsequent productions.

TABLE 18

Correlation surface for percentage of butter fat in the eight-months milk production at three years old and percentage of butter fat in the eight-months milk production at six years old for pure-bred Jersey cows.

6 years	3 years												
	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	Total
4.00-4.20	1												1
4.20-4.40		1	2										3
4.40-4.60	1		1	1				1					4
4.60-4.80		1	3		2	1	1						8
4.80-5.00		1	6	1	4	4	1						17
5.00-5.20		1	1	5	5	4	2	2					20
5.20-5.40	1		2		4	3	7	4		1			22
5.40-5.60				2	3	4	4	2	3	1			19
5.60-5.80					1	3	2		2				8
5.80-6.00							1	1	1				3
6.00-6.20					1	1							2
6.20-6.40												1	1
Total	3	4	15	9	20	20	18	10	6	2	0	1	108

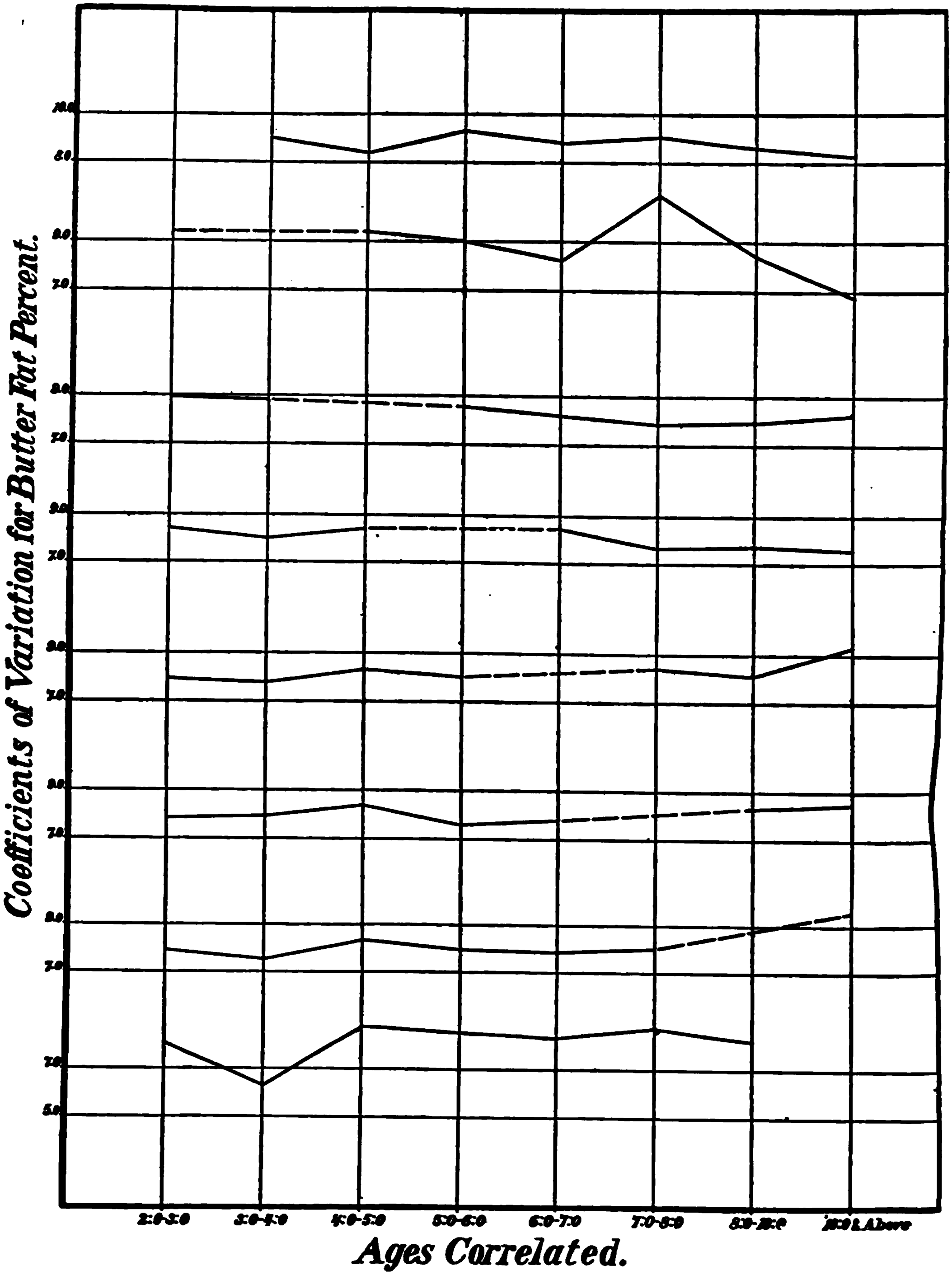

FIGURE 6.—Curves showing the standard deviations of the butter-fat percentage of the milk of a given age in the cow's life at the successive ages with which the given age is correlated. The ordinates represent the standard deviations of the butter-fat percentages which are produced. The abscissae are the ages of the groups with which the given age is correlated and for which the given age had the standard deviation plotted. The curves proceeding from the top to the bottom of the figure are for the ages, two, three, four, five, six, seven, eight and nine, and ten years and older. The lines are quite regular and in general are parallel with the base as would be expected if no selection were practiced.

Two curves only show any considerable variation. These curves are for the ages three years to four years and ten years and older. This variation considered mathematically is no doubt significant; but if the relatively few individuals in the class and the general trend of the rest of the curve are taken into consideration much of this significance is lost. The other curves are all parallel with the base of the figure and are in accord in showing that no selection of high-butter-fat-testing cows to be retained in the herd for milkers at the older ages based on their earlier recorded production, has been practiced.

TABLE 19

Correlation surface for percentage of butter fat in the eight-months milk production at three years old and percentage of butter fat in the eight-months milk production at seven years old for pure-bred Jersey cows.

3 years

7 years	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	6.60-6.80	6.80-7.00	Total
4.00-4.20	1		1												2
4.20-4.40	2														2
4.40-4.60		1		1	3										5
4.60-4.80			2	1		1		1							5
4.80-5.00	1	1	2	2	5	1	6	2		1					21
5.00-5.20		2	2	3	2	6	3								18
5.20-5.40		1	3	1	4	4	5	4							22
5.40-5.60				1	3	4	4	1	2						15
5.60-5.80							1								1
5.80-6.00						1		1		1					3
6.00-6.20			1			1				1				1	4
Total	4	5	11	9	17	18	19	9	2	3	0	0	0	1	98

TABLE 20

Coefficients of variation for butter-fat percent of a given year when correlated with other given years.

Ages with which correlated	Age correlated			
	2.0-3.0	3.0-4.0	4.0-5.0	5.0-6.0
2.0- 3.0	————	9.395 ± .342	8.956 ± .348	8.461 ± .350
3.0- 4.0	9.008 ± .327	————	8.808 ± .347	8.009 ± .332
4.0- 5.0	8.457 ± .326	9.391 ± .370	————	8.405 ± .344
5.0- 6.0	9.332 ± .386	8.992 ± .374	8.562 ± .350	————
6.0- 7.0	8.803 ± .396	8.181 ± .369	8.207 ± .371	8.391 ± .359
7.0- 8.0	9.060 ± .459	10.932 ± .536	7.867 ± .368	7.653 ± .341
8.0-10.0	8.665 ± .427	8.426 ± .373	7.951 ± .355	7.739 ± .308
10.0 and above	8.292 ± .693	6.660 ± .770	8.253 ± .539	7.574 ± .485

TABLE 20 (continued)

Coefficients of variation for butter-fat percent of a given year when correlated with other given years.

Ages with which correlated	Age correlated			
	6.0-7.0	7.0-8.0	8.0-10.0	10.0 and above
2.0- 3.0	7.933 ± .357	7.807 ± .397	7.894 ± .389	8.120 ± .679
3.0- 4.0	7.764 ± .369	7.866 ± .388	7.493 ± .326	6.297 ± .659
4.0- 5.0	8.295 ± .375	8.322 ± .390	8.299 ± .368	8.782 ± .574
5.0- 6.0	8.040 ± .342	7.540 ± .336	7.902 ± .315	8.601 ± .552
6.0- 7.0	———	7.757 ± .337	7.812 ± .309	8.411 ± .526
7.0- 8.0	8.418 ± .366	———	7.959 ± .310	8.795 ± .498
8.0-10.0	8.137 ± .322	8.237 ± .320	———	8.323 ± .345
10.0 and above	9.341 ± .585	8.410 ± .476	9.377 ± .389	———

The coefficients of variation of milk production as influenced by the time the cow is retained in the herd

The data for these constants are taken from the correlation tables 7 to 41. Table 20 gives the coefficients of variation for these distributions. Their arrangement is identical with that for tables 9 and 17. The age for which the coefficients of variation are determined is given above the columns. The ages at which the cows have records at other ages are given at the left of the table.

The data in this table have been made into curves for each age as seen in figure 7.

The values of the coefficients of variation are represented as ordinates and the ages at which the cows had another lactation as abscissae. The curves have the same arrangement as in figures 5 and 6. The same reasoning,—that if no selection of cows based on their previous records has taken place the curves generated by these coefficients of variation should be straight lines parallel with the base,—still holds for this figure.

The curves of figure 7 are clearly parallel with the base, save for a noticeable drop in the coefficient of variation occurring in the ten-year curve at three years.

For the ten-year group the difference is considerably larger than three times its error. In the ten-year curve, however, the subsequent coefficients of variation are nearly equal to those preceding this drop. This, of course, would not be expected if the drop were due to selection having taken place at the point of the low coefficient, for if it had taken place

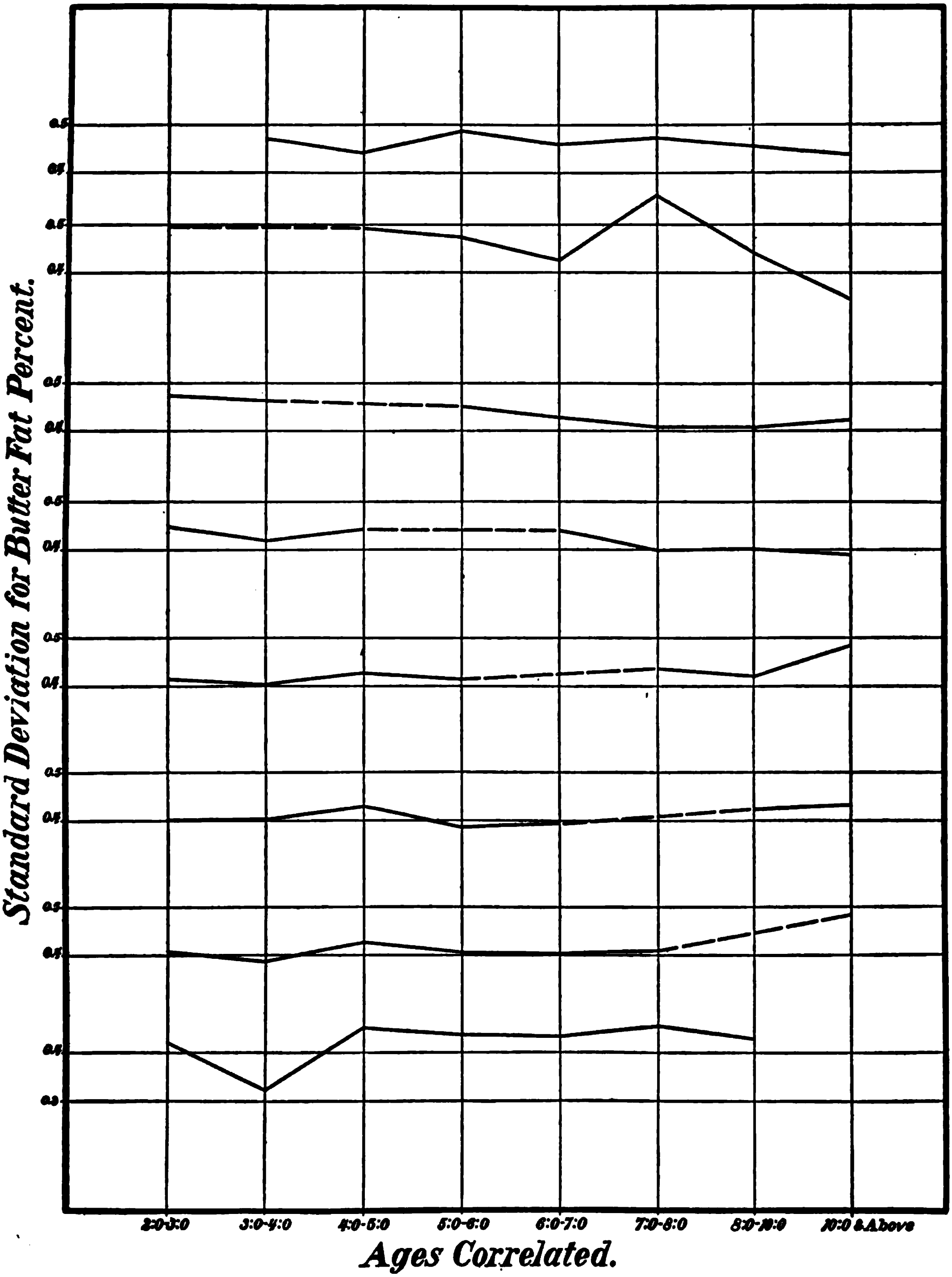

FIGURE 7.—Curves showing the coefficients of variation for the butter-fat percentage of a given age at each successive age with which the given age is correlated. The ordinates are the coefficients of variation and the abscissae are the ages of the groups with which the given age is correlated. The significance of the curves from top to bottom of the figure is the same as in figures 5 and 6. The lines generated by the curves should, like the preceding curves, be parallel with the base if no selection has taken place.

TABLE 21

Correlation surface for percentage of butter fat in the eight-months milk production at three years old and percentage of butter fat in the eight-months production at eight and nine years old for pure-bred Jersey cows.

3 years

8 and 9 years	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	6.60-6.80	6.80-7.00	Total
4.20-4.40	1		1				1								3
4.40-4.60	1	1	3			1		2							8
4.60-4.80		2	2	1	1		1			1					8
4.80-5.00	1			1		2	4	1	1						10
5.00-5.20	1		2	2	7	5	1	2							20
5.20-5.40	1		1	2	7	5	6	3							25
5.40-5.60				2	3	7	1	5	2	1					21
5.60-5.80				1	1		2	2		1					7
5.80-6.00						2	1								3
6.00-6.20															0
6.20-6.40														1	1
Total	5	3	9	9	19	22	17	15	3	3	0	0	0	1	106

TABLE 22

Correlation surface for percentage of butter fat in the eight-months milk production at three years old and percentage of butter fat in the eight-months production at ten-and-above years old for pure-bred Jersey cows.

3 years

10 years and older	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	Total
4.40-4.60	1									1
4.60-4.80						1				1
4.80-5.00			1	3		1	1			6
5.00-5.20							1			1
5.20-5.40						1	1			2
5.40-5.60					4	2	1		1	8
Total	1	0	1	3	4	5	3	0	1	19

the coefficients should be low for the rest of the life of this group of cows. For our problem then this irregularity in the coefficients of variation would seem of little significance.

The conclusion which may be drawn from this study of the means, standard deviations and coefficients of variation is that no selection of cows for future milkers on the basis of their butter-fat percentage in previous lactations has been practiced at any time in the herd's history.

TABLE 23

Correlation surface for percentage of butter fat in the eight-months milk production at four years old and percentage of butter fat in the eight-months milk production at five years old for pure-bred Jersey cows.

5 years old	4 years old: 4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	6.60-6.80	6.80-7.00	Total
4.00-4.20			1	1											2
4.20-4.40															0
4.40-4.60	1	1	2	1											5
4.60-4.80			4	2	4	3	1								14
4.80-5.00	1		1	8	6	1									17
5.00-5.20			2	6	5	7	1	3		1	1				26
5.20-5.40		1	1	5	3	6	4	1	1						22
5.40-5.60			1	1	1	11	6	4	1						25
5.60-5.80				1	1	2	2	4	1	1	1			1	14
5.80-6.00								5	3						8
6.00-6.20							1			1					2
6.20-6.40									1						1
6.40-6.60															0
6.60-6.80											1			1	2
Total	2	2	12	25	20	30	15	17	7	3	3	0	0	2	138

TABLE 24

Correlation surface for percentage of butter fat in the eight-months milk production at four years old and percentage of butter fat in the eight-months milk production at six years old for pure-bred Jersey cows.

6 years old	4 years old: 4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20			1									1
4.20-4.40		1	1	2								4
4.40-4.60	1	1	2	1	1				1			7
4.60-4.80	1	1	4	1	2	2						11
4.80-5.00			3	4	1	4	3	2				17
5.00-5.20				3	4	3	4	2		1		17
5.20-5.40		1	2	3	3	10	3	2			1	25
5.40-5.60				3	3	3	2	3	1	1	1	17
5.60-5.80				1	4	1	1	1	1			9
5.80-6.00								1				1
6.00-6.20								1			1	2
6.20-6.40								1		1		2
Total	2	4	13	18	18	23	13	13	3	3	3	113

This important conclusion regarding the data reflects back on the conclusion drawn from the studies of the earlier sections of the paper as those conclusions are freed from the one possible criticism that selection of the best-producing animals, to be kept for the milkers in later life, by the records made while they were young, has materially influenced the general applicability of the results of this study to the breed of Jersey cattle as a whole.

The correlation of the butter-fat percentage for eight-months milk yield at a given age with the like butter-fat percentage at any other given age

The homogeneous nature of the records established by the previous analysis, the data may now be used for the correlations themselves, knowing that the data on which these correlations are based are such that the values of the correlations are their true value. The tables on which these correlation coefficients are determined are found in tables 7 to 41. The means, standard deviations and coefficients of variation are given in tables 9, 17 and 20. Table 25 gives the correlations and their probable errors for all ages at which the lactation records were divided. The vertical columns as in the preceding tables for the other constants of the correlation surfaces give the correlations of the butter-fat percentage of the ages heading the column with the butter-fat percentage at the ages indicated on the left-hand margin of the table. As will be noted the correlations necessary to give the complete set of correlations for any age are repeated, e.g., the correlation of 2-years butter-fat percentage with that at 3 years is + 0.5277 ± .0368 and appears in

TABLE 25

Coefficients of correlation for butter-fat percent of a given age when correlated with other given ages.

Age with which correlated	Age correlated			
	2.0-3.0	3.0-4.0	4.0-5.0	5.0-6.0
2.0- 3.0	—	+ 0.5277 ± .0368	+ 0.5288 ± .0390	+ 0.5846 ± .0382
3.0- 4.0	+ 0.5277 ± .0368	—	+ 0.6071 ± .0349	+ 0.5836 ± .0384
4.0- 5.0	+ 0.5288 ± .0390	+ 0.6071 ± .0349	—	+ 0.6781 ± .0310
5.0- 6.0	+ 0.5846 ± .0382	+ 0.5836 ± .0384	+ 0.6781 ± .0310	—
6.0- 7.0	+ 0.5956 ± .0407	+ 0.5861 ± .0426	+ 0.5236 ± .0460	+ 0.5529 ± .0417
7.0- 8.0	+ 0.6068 ± .0452	+ 0.2470 ± .0640	+ 0.5668 ± .0447	+ 0.4830 ± .0480
8.0-10.0	+ 0.5695 ± .0468	+ 0.4311 ± .0533	+ 0.4475 ± .0498	+ 0.5638 ± .0382
10.0 and above	+ 0.5739 ± .0787	+ 0.5250 ± .1121	+ 0.5163 ± .0674	+ 0.4138 ± .0747

TABLE 25 (continued)

Coefficients of correlation for butter-fat percent of a given age when correlated with other given ages.

Age with which correlated	Age correlated			
	6.0-7.0	7.0-8.0	8.00-10.0	10.0 and above
2.0- 3.0	+ 0.5956 ± .0407	+ 0.6068 ± .0452	+ 0.5695 ± .0468	+ 0.5739 ± .0787
3.0- 4.0	+ 0.5861 ± .0426	+ 0.2470 ± .0640	+ 0.4311 ± .0533	+ 0.5250 ± .1121
4.0- 5.0	+ 0.5236 ± .0460	+ 0.5668 ± .0447	+ 0.4475 ± .0498	+ 0.5163 ± .0674
5.0- 6.0	+ 0.5529 ± .0417	+ 0.4830 ± .0480	+ 0.5638 ± .0382	+ 0.4138 ± .0747
6.0- 7.0	———	+ 0.5594 ± .0420	+ 0.4678 ± .0434	+ 0.3196 ± .0788
7.0- 8.0	+ 0.5594 ± .0420	———	+ 0.6004 ± .0349	+ 0.4137 ± .0659
8.0-10.0	+ 0.4678 ± .0434	+ 0.6004 ± .0349	———	+ 0.5294 ± .0436
10.0 and above	+ 0.3196 ± 0.0788	+ 0.4137 ± .0659	+ 0.5294 ± .0436	———

the two-year column on line with the three-year age. The correlation of the three-years butter-fat percentage with that of the two-years will, of course, be the same (0.5277 ± .0368) and is repeated in the three-year column on the line with the two-year age. Such an arrangement facilitates the grasping of the complete picture of the relation between the yield of a given age and that of any other age, as each column represents the correlation coefficients of that year with the other years.

The largest of these correlation coefficients for the butter-fat percentage of one lactation in comparison with that of another is + 0.6781 ± .0310 for the lactation at four years old and at five years old. The lowest correlation coefficient is 0.2470 ± .0640 for the comparison of the butter-fat percentage of the three-year-olds with that of the seven-year-olds. All of these correlations are plus. There was no correlation out of the fifty-six determined which was not significant. Such high correlations point to a regulatory mechanism behind the mammary function, which governs, within certain limits, the concentration of butter fat which a given cow is able to secrete into her milk from one lactation to another.

The graphs showing these correlations for each year are seen in figure 8. The arrangement of the figure is entirely similar to that of figures 5, 6 and 7. The sizes of the correlations are represented as ordinates and the ages of the cows whose butter-fat percentages are correlated with those of the given age are the abscissae. The curves progressing from the top to the bottom of the figure represent the correla-

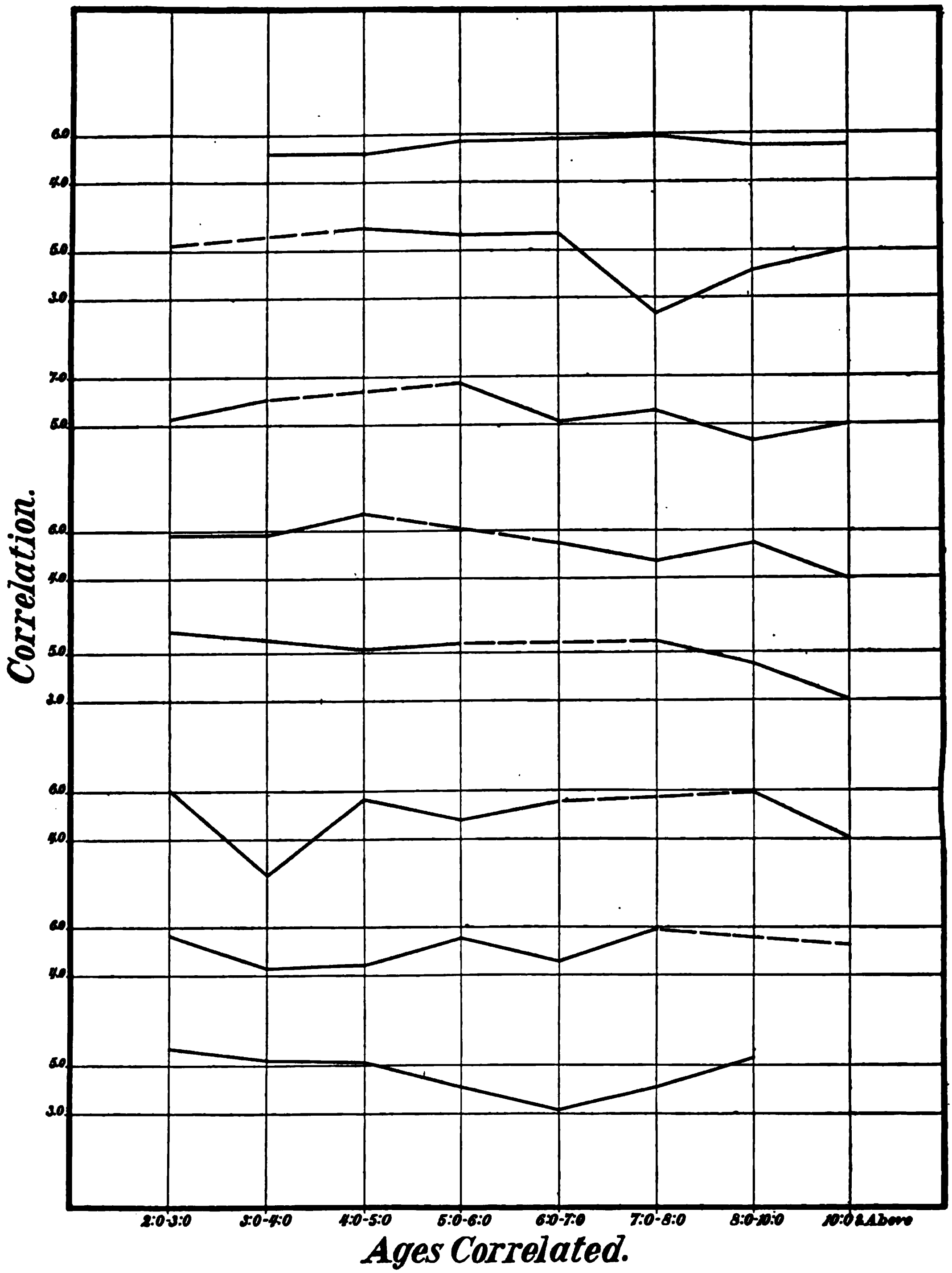

FIGURE 8.—Curves showing the coefficients of correlation of butter-fat percentage of a given age and the butter-fat percentage at any other given age. The ordinates are the values of the correlation coefficients. The abscissae are the ages with which the butter-fat percentage of the given cow is correlated. For example the top curve of the figure shows the correlation of the butter-fat percentage made at two years with the records made at subsequent ages. From this curve the correlation between the butter-fat percentage made at two years old and the butter-fat percentage made at three years old is read off on the three-year vertical of the two-year line (top curve of figure) as 0.5277. The correlation of the two-year butter-fat percentage and the four-year butter-fat percentage made by the same cows at these ages is seen on the four-year vertical, two-year line to be 0.5288. The dotted lines serve to connect the correlations immediately preceding and following the age for which the given age line is drawn.

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tions for the butter-fat percentage at the ages two, three, four, five, six, seven, eight and nine and ten-years-and-older with the other butter-fat percentages occurring at the ages shown on the lower margin of the figure.

These curves are, on the whole, linear in nature and parallel with the base of the figure. In other words the correlations of butter-fat percentage of a given-aged cow with that of the other ages at which this cow may have another lactation records are approximately of the same values throughout.

Two possible exceptions to this conclusion may be pointed out. These two possible exceptions depend on the one correlation (0.2470 ± .0646) for the correlations of the butter-fat percentages at the ages three and seven years. It is true that this drop in each of these curves does not fit in the general curve as well as might be desired. The significance of the difference from the general level of the curves is only slight if significant at all.

TABLE 26

Correlation surface for percentage of butter fat in the eight-months milk production at four years old and percentage of butter fat in the eight-months milk production at seven years old for pure-bred Jersey cows.

	4 years old											
7 years old	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20		1										1
4.20-4.40	1	1	2			1						5
4.40-4.60		1		2		2						5
4.60-4.80		1	2	2	1	1						7
4.80-5.00		1	2	6	3		1	1	1		1	16
5.00-5.20			3	6	5	5	2	1				22
5.20-5.40				3	5	6	4	3		1		22
5.40-5.60		1		3		7		4				15
5.60-5.80					2		1		1			4
5.80-6.00								2				2
6.00-6.20						1		2	2		1	6
Total	1	6	9	22	16	23	8	13	4	1	2	105

The average level of the correlations for butter-fat percentage of a lactation at a given age with those at any other age is of especial interest to the dairyman since the size of the correlation is the index by which he may choose the lactation on which to base the selection of animals to remain in the herd as future milkers. The averages of these corre-

TABLE 27

Correlation surface for percentage of butter fat in the eight-months milk production at four years old and percentage of butter fat in the eight-months milk production at eight and nine years old for pure-bred Jersey cows.

4 years old

8 and 9 years old	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.20-4.40	1		1	2			1					5
4.40-4.60	2	2	3	2	1	1						11
4.60-4.80		1		4	2		2				1	10
4.80-5.00		1	1	2	3	3	2	1				13
5.00-5.20			1	7	6	4		1	1			20
5.20-5.40		2	3	5	5	2		2	1			20
5.40-5.60		1		2	3	11	1	3	1	2		24
5.60-5.80				1	2	2		2	1			8
5.80-6.00					1	1		1	1			4
6.00-6.20								1				1
6.20-6.40									1			1
Total	3	7	9	25	23	24	6	11	6	2	1	117

TABLE 28

Correlation surface for percentage of butter fat in the eight-months milk production at four years old and percentage of butter fat in the eight-months milk production of ten-and-above years old for pure-bred Jersey cows.

4 years old

10-and-above years old	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20			1									1
4.20-4.40		1		1								2
4.40-4.60	1			2								3
4.60-4.80		1		1								2
4.80-5.00			1	2		1	2					6
5.00-5.20		1		3		2		1			1	8
5.20-5.40			2	1	2	4		1	1	1		12
5.40-5.60				4	3	1		1				9
5.60-5.80						1		1	1			3
5.80-6.00												0
6.00-6.20						1		2				3
Total	1	3	4	14	5	10	2	6	2	1	1	49

lations have accordingly been made. The highest average correlation coefficient is for the butter-fat percentage of the lactation commencing between the ages two years to three years (0.5696). The next highest average correlation coefficient is for the four year age (0.5520). The five-year age is third (0.5514), the eight-and-nine-year age is fourth (0.5156). The other ages at lactation follow in the order, six, three, seven and ten-and-older years. The differences in these correlations are of only doubtful significance so that no conclusion as to the relative merit of the use of one lactation over that of another as a basis for selection of animals to remain in the herd, can with certainty be made. Further from the theoretical side no conclusion can be drawn from these figures as to any differential action of the mechanism or effect of environment on the milk production and butter-fat percentage at these different ages. They do, however, lead to the important practical conclusion that a cow commencing her lactations as a two-year-old with a high butter-fat percentage may be expected to duplicate this relatively high performance within a small error in the next and succeeding lactations. The first lactation records as to the butter-fat percentage that a given cow will produce are, then, a good index of what may be expected in future years of that cow. As will be shown in a subsequent section, selection of cows to remain in the herd on the basis of these records is profitable to the dairyman.

TABLE 29

Correlation surface for percentage of butter fat in the eight-months milk production at five years old and percentage of butter fat in the eight-months milk production at six years old for pure-bred Jersey cows.

6 years old	5 years old 4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00 6.20	6.20-6.40	6.40-6.60	6.60-6.80	Total
4.00-4.20							2								2
4.20-4.40				1		1	2								4
4.40-4.60	2		1	1			1								5
4.60-4.80			1	6	2	2		1							12
4.80-5.00				2	4	3	1	3	1						14
5.00-5.20			2	3	4	8	1	4	1	1	1				25
5.20-5.40			1	1	4	6	4	8	1	2					27
5.40-5.60					1	2	7	3	3	5					21
5.60-5.80					1		1	4	3	1					10
5.80-6.00								1				1			2
6.00-6.20							1			1				1	3
6.20-6.40											1				1
Total	2	0	5	14	16	22	20	24	9	10	2	1	0	1	126

The comparison of these correlations with those on milk production for the same data, using the same divisions, is of considerable interest as

TABLE 30

Correlation surface for percentage of butter fat in the eight-months milk production at five years old and percentage of butter fat in the eight-months milk production at seven years old for pure-bred Jersey cows.

5 years old

7 years old	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	6.60-6.80	Total
4.00-4.20	1												1
4.20-4.40	1				1								2
4.40-4.60	1	1	1	2	1	1							7
4.60-4.80	1	2	1	4	1								9
4.80-5.00		2	3	4	7	1	3						20
5.00-5.20	1	5	1	7	5	6							25
5.20-5.40	1	2	4	6	4	3	4	1	1				26
5.40-5.60			2	2	5	4	2	1		1			17
5.60-5.80									1				1
5.80-6.00					2		1	1					4
6.00-6.20				1			1	1				1	4
Total	6	12	12	26	26	15	11	4	2	1	0	1	116

showing the relative strength by which one lactation governs the future production of another lactation. Table 31 gives this comparison for the average unweighted coefficients of correlation for the records of each age with the records made at another age.

These correlation coefficients range in value from 0.4702 to .5696 for the butter-fat percentage and from 0.4597 to .5694 for the milk pro-

TABLE 31

Average coefficients of correlation for lactation records made at a given age and lactation records made at other ages for milk production and butter-fat percentage.

Age at which given record is made	Coefficient of correlation		Difference, butter-fat percentage less milk production
	Butter-fat percentage	Milk production	
2 years to 3 years..........	+ 0.5696	0.5491	.0205
3 years to 4 years..........	.5011	.5694	— .0683
4 years to 5 years..........	.5526	.5060	.0466
5 years to 6 years..........	.5514	.5735	— .0221
6 years to 7 years..........	.5150	.5661	— .0511
7 years to 8 years..........	.4967	.5501	— .0534
8 years to 10 years..........	.5156	.5077	.0079
10 years and older............	.4702	.4597	.0105
Average of records at all ages	.5215	.5352	— .0137

duction. The average value of the butter-fat percentage correlation coefficients is 0.5215 and the average value of the milk yields is .0352.

Of the sixteen average coefficients of correlation four of those for the butter-fat percentage are higher than those for the milk yield and four of them are lower.

The greatest difference of these coefficients is — 0.0683. The differences of the average values is — .0137. From the numbers involved it seems probable that these differences are so small as not to be significant. Such being the case it follows that the relative accuracy in the use of one lactation record to predict the expected record of another lactation is approximately the same for the butter-fat percentage and for the milk yield. In other words the governing power (presumably the complex given the animal through its inherited factors for these two characters) works with about the same accuracy (as measured by its performance) from lactation to lactation. This would by no means necessarily mean that the inheritance of these two characters is the same, in fact in all probability high milk production is governed more by dominant factors than is high butter-fat percentage (GOWEN 1918). It only means that these factors once given an animal hold it to the same relative level from lactation to lactation.

TABLE 32

Correlation surface for percentage of butter fat in the eight-months milk production at five years old and percentage of butter fat in the eight-months milk production at eight and nine years old for pure-bred Jersey cows.

8 and 9 years old	5 years old 4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.20-4.40			1	2		2		1					6
4.40-4.60	1		2	4	2	1	1	1					12
4.60-4.80			1	2	2	1	2	1					9
4.80-5.00	1		1	2	3	7	4	2	1				21
5.00-5.20				3	3	6	7	2					21
5.20-5.40			1	1	7	7	5	9	1				31
5.40-5.60				1	2	1	6	7	4	3		1	25
5.60-5.80					1	1	2	1	4	2	1	1	13
5.80-6.00						1	2		2	1	1		7
Total	2	0	6	15	20	27	29	24	12	6	2	2	145

TABLE 33

Correlation surface for percentage of butter fat in the eight-months milk production at five years old and percentage of butter fat in the eight-months milk production at ten-and-above years old for pure-bred Jersey cows.

5 years old

10-and-above years old	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20		1									1
4.20-4.40	1	1		2							4
4.40-4.60	1			2							3
4.60-4.80	1		1	1	1						4
4.80-5.00		1		4	5						10
5.00-5.20	2		1	3	2					1	9
5.20-5.40		1	1	3	1	2	2		1		11
5.40-5.60		2		2	1	4					9
5.60-5.80				1					1		2
5.80-6.00											0
6.00-6.20				1							1
6.20-6.40							1				1
Total	5	6	3	19	10	6	3	0	2	1	55

TABLE 34

Correlation surface for percentage of butter fat in the eight-months milk production at six years old and percentage of butter fat in the eight-months milk production at seven years old for pure-bred Jersey cows.

6 years old

7 years old	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	Total
4.00-4.20		1	1		1									3
4.20-4.40	1						1							2
4.40-4.60		1		3	1	1								6
4.60-4.80			2	3	2	4	1	1			1			14
4.80-5.00		3	1	2.	4		7	2	4					23
5.00-5.20				3	4	3	7	3	1	1				22
5.20-5.40				1	4	12	4	6	1					28
5.00-5.60							5	5	2	2			1	15
5.60-5.80				1				1	1					3
5.80-6.00								2			1			3
6.00-6.20								1			1	1		3
Total	1	5	4	13	16	20	25	21	9	3	3	1	1	122

If we transfer our reasoning to the race of Jersey cattle with which we are dealing these records of the individuals in this race show a distinct differentiation. The high individuals tend to remain high, the low individuals low, with respect to their quality of butter-fat percentage,

TABLE 35

Correlation surface for percentage of butter fat in the eight-months milk production at six years old and percentage of butter fat in the eight-months milk production at eight and nine years old for pure-bred Jersey cows.

8 and 9 years old \ 6 years old	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	6.40-6.60	Total
3.80-4.00						1								1
4.00-4.20														0
4.20-4.40			1		3									4
4.40-4.60		1	4	4	1									10
4.60-4.80	1			4	3	2	2	1						13
4.80-5.00			1	3	3	2	3	6						18
5.00-5.20				3	6	5	3	6			2			25
5.20-5.40			1	3	4	7	7	3	4	2				31
5.40-5.60					6	3	7	8	3	1		1	1	30
5.60-5.80					1		4	1	2	1				9
5.80-6.00							1	1			1			3
6.00-6.20				2								1		3
Total	1	1	7	19	27	20	27	26	9	4	3	2	1	147

just as they also do with respect to their quantity of milk. Such a difference can mean but one thing: The animals in this race are innately differentiated with regard to their capacity to secrete a high concentration of butter fat into their milk as well as they are for the capacity to secrete the quantity of milk.

Only one other economic product has been dealt with quantitatively by the correlation method (HARRIS and BLAKESLEE 1918). The correlation coefficients in this case deal with the relation of the monthly egg production to the egg production of the other eleven months of the year. The correlations for these ovulation records range from + 0.240 ± .033 to + .573 ± .023. The range for the correlations of butter-fat percentage is + 0.2470 ± .0680 to + 0.6781 ± .0310. The range in these butter-fat percentage correlations is greater than that for the ovulation records of the White Leghorn hen. The mean coefficient of correlation for these ovulation records is 0.446. This mean coefficient of correlation is, consequently, slightly below that for butter-fat percentage (.5215), the difference being .0755. This difference, on the face of it, would seem to indicate that a greater dependence may be placed in the record of the butter-fat percentage of a known lactation as to the future butter-fat percentage in a given cow's milk than can be placed in a knowledge of a month's egg production to determine the future production of the hen. The difference is not great, however, and may not be statistically significant.

THE REGRESSION OF THE BUTTER-FAT PERCENTAGE OF LACTATIONS AT DIFFERENT AGES ONE ON THE OTHER

The constants necessary to determine the approach of the regression lines to linearity for the correlation coefficients of table 25 are given in table 36. The values of the correlation coefficients have been repeated for greater ease of comparison.

In this table the correlation ratios range in value from 0.3992 ± .0573 to 0.7374 ± .0707. These values of the correlation ratios are somewhat higher than are the values for the correlation coefficients as may be seen in table 25. The difference between the correlation ratios and the correlation coefficients are not significant as measured by the probable error of this difference. The regression of the butter-fat percentage at one age on that of another diverges only slightly from the linear as may be seen by the relation of $\eta^2 - r^2$ to its probable error. These values range between 2.98 to .96 with a mean value of 2.14. Such a small range together with the low mean value makes it likely that the regressions of all of these curves are linear. The values of the correlation coefficients can, therefore, be considered as correct in measuring the association of the butter-fat percentage of one lactation on another.

From these correlations of tables 25 and 36 it is possible to form the straight-line predictions of the butter-fat percentage for any age from the butter-fat percentage of any other age. The general equations for these regressions are given by

$$B = \text{Mean}_B - r_{Bb} \frac{\sigma_B}{\sigma_b} \text{Mean}_b + r_{Bb} \frac{\sigma_B}{\sigma_b} b,$$

when B is the expected butter-fat percentage at the age desired and b is the butter-fat percentage which is given. The results of the substitution of the value contained in tables 9, 17 and 25 are given in table 37.

The use of these equations is merely a matter of substitution. Suppose that the butter-fat percentage of a cow is equal to 5.34 when she is two years old, what will be her expected percentage at six years old? The equation for this age is

$$B = 2.422 + .535b_2$$

substituting for b_2 the value of the butter-fat percentage actually obtained (5.34) we have 5.279 as the expected butter-fat percentage of this cow

TABLE 36

Analytical constants for the linearity of regression of butter-fat percentage of one lactation on another lactation.

Character	r	η	$\eta - r$	$\eta^2 - r^2$	$\frac{\eta^2 - r^2}{E_{\eta^2 - r^2}}$
2 years to 3 years...........	+ 0.5277 ± .0368	+ 0.5673 ± .0346	0.0396 ± .0505	0.0434 ± .0209	2.08
2 " " 4 "	.5288 ± .0390	.5833 ± .0358	.0545 ± .0530	.0606 ± .0258	2.35
2 " " 5 "	.5846 ± .0382	.6373 ± .0345	.0527 ± .0515	.0644 ± .0285	2.26
2 " " 6 "	.5956 ± .0407	.6077 ± .0398	.0120 ± .0569	.0146 ± .0151	.96
2 " " 7 "	.6068 ± .0452	.6591 ± .0404	.0523 ± .0606	.0662 ± .0355	1.86
2 " " 8 and 9 years.....	.5695 ± .0468	.6323 ± .0416	.0628 ± .0626	.0755 ± .0366	2.07
2 " " 10 and older.......	.5739 ± .0787	.7278 ± .0552	.1539 ± .0961	.2003 ± .0940	2.13
3 years to 4 years...........	.6071 ± .0349	.6829 ± .0295	.0758 ± .0457	.0978 ± .0328	2.98
3 " " 5 "	.5836 ± .0384	.6449 ± .0340	.0613 ± .0513	.0753 ± .0308	2.45
3 " " 6 "	.5861 ± .0426	.6221 ± .0398	.0360 ± .0583	.0435 ± .0265	1.64
3 " " 7 "	.2470 ± .0640	.3992 ± .0573	.1522 ± .0859	.0984 ± .0406	2.42
3 " " 8 and 9 years......	.4311 ± .0533	.5382 ± .0465	.1072 ± .0708	.1039 ± .0400	2.60
3 " " 10 and older.......	.5250 ± .1121	.7374 ± .0707	.2125 ± .1325	.2681 ± .1372	1.95
4 years to 5 years...........	.6781 ± .0310	.7175 ± .0278	.0395 ± .0417	.0550 ± .0262	2.10
4 " " 6 "	.5236 ± .0460	.5791 ± .0422	.0555 ± .0624	.0612 ± .0304	2.01
4 " " 7 "	.5668 ± .0447	.5996 ± .0421	.0328 ± .0614	.0383 ± .0252	1.52
4 " " 8 and 9 years......	.4475 ± .0498	.5283 ± .0450	.0807 ± .0671	.0788 ± .0336	2.35
4 " " 10 and older.......	.5163 ± .0674	.6485 ± .0532	.1322 ± .0859	.1540 ± .0663	2.32
5 years to 6 years...........	.5529 ± .0417	.6433 ± .0352	.0904 ± .0546	.1081 ± .0373	2.90
5 " " 7 "	.4830 ± .0480	.5399 ± .0444	.0569 ± .0654	.0582 ± .0293	1.99
5 " " 8 and 9 years......	.5638 ± .0382	.5979 ± .0360	.0341 ± .0525	.0396 ± .0218	1.81
5 " " 10 and older.......	.4138 ± .0747	.4895 ± .0685	.0757 ± .1013	.0684 ± .0455	1.50
6 years to 7 years...........	.5594 ± .0420	.6014 ± .0390	.0420 ± .0573	.0488 ± .0263	1.85
6 " " 8 and 9 years......	.4678 ± .0434	.5467 ± .0390	.0789 ± .0584	.0800 ± .0302	2.65
6 " " 10 and older.......	.3196 ± .0788	.4761 ± .0679	.1565 ± .1040	.1245 ± .0580	2.15
7 years to 8 and 9 years......	.6004 ± .0349	.6589 ± .0308	.0585 ± .0466	.0737 ± .0285	2.59
7 " " 10 and older.......	.4137 ± .0659	.5089 ± .0589	.0952 ± .0884	.0878 ± .0450	1.95
8 and 9 years to 10 and older	.5294 ± .0436	.5973 ± .0390	.0679 ± .0585	.0765 ± .0322	2.38

TABLE 37

Regression equations of the butter-fat percentage for any age from that on any lactation record at another age.

Age at which butter-fat percentage was made	Age at which expected butter-fat percentage is desired 2 years to 3 years Regression equation	3 years to 4 years Regression equation	4 years to 5 years Regression equation	5 years to 6 years Regression equation
2 years to 3 years b_2	$B =$ ————	$B = 2.376 + .556b_2$	$B = 2.340 + .569b_2$	$B = 2.482 + .534b_2$
3 years to 4 years b_3	$B = 2.665 + .501b_3$	$B =$ ————	$B = 2.269 + .570b_3$	$B = 2.525 + .518b_3$
4 years to 5 years b_4	$B = 2.743 + .492b_4$	$B = 1.871 + .646b_4$	$B =$ ————	$B = 1.757 + .662b_4$
5 years to 6 years b_5	$B = 1.925 + .640b_5$	$B = 1.789 + .658b_5$	$B = 1.588 + .694b_5$	$B =$ ————
6 years to 7 years b_6	$B = 1.747 + .663b_6$	$B = 1.954 + .621b_6$	$B = 2.414 + .525b_6$	$B = 2.114 + .584b_6$
7 years to 8 years b_7	$B = 1.367 + .719b_7$	$B = 3.362 + .345b_7$	$B = 2.361 + 539b_7$	$B = 2.542 + .498b_7$
8 years to 10 years b_8	$B = 1.789 + .639b_8$	$B = 2.602 + .491b_8$	$B = 2.945 + .429b_8$	$B = 2.265 + .557b_8$
10 years and older b_{10}	$B = 2.007 + .599b_{10}$	$B = 2.289 + .556b_{10}$	$B = 2.660 + .484b_{10}$	$B = 3.190 + .359b_{10}$

TABLE 37 (continued)

Regression equations of the butter-fat percentage for any age from that on any lactation record at another age.

Age at which butter-fat percentage was made	Age at which expected butter-fat percentage is desired 6 years to 7 years Regression equation	7 years to 8 years Regression equation	8 years to 10 years Regression equation	10 years and older Regression equation
2 years to 3 years b_2	$B = 2.422 + .535b_2$	$B = 2.452 + .512b_2$	$B = 2.485 + .507b_2$	$B = 2.268 + .550b_2$
3 years to 4 years b_3	$B = 2.304 + .554b_3$	$B = 4.232 + .177b_3$	$B = 3.196 + .378b_3$	$B = 2.606 + .495b_3$
4 years to 5 years b_4	$B = 2.427 + .522b_4$	$B = 2.066 + .596b_4$	$B = 2.748 + .467b_4$	$B = 2.321 + .550b_4$
5 years to 6 years b_5	$B = 2.435 + .523b_5$	$B = 2.698 + .468b_5$	$B = 2.193 + .571b_5$	$B = 2.702 + .464b_5$
6 years to 7 years b_6	$B =$ ————	$B = 2.473 + .507b_5$	$B = 2.846 + .448b_6$	$B = 3.664 + .284b_6$
7 years to 8 years b_7	$B = 1.899 + .617b_7$	$B =$ ————	$B = 2.162 + .580b_7$	$B = 2.936 + .435b_7$
8 years to 10 years b_8	$B = 2.633 + .489b_8$	$B = 1.953 + .621b_8$	$B =$ ————	$B = 2.733 + .469b_8$
10 years and older b_{10}	$B = 3.275 + .359b_{10}$	$B = 3.145 + .394b_{10}$	$B = 2.069 + .598b_{10}$	$B =$ ————

for her lactation at six years. Similarly the expected butter-fat percentage of a lactation at any age may be predicted from a record at any other age. The only limiting factor for these equations is that the lactation records be for a period of eight months. As the equations are given for actual butter-fat percentage the second term in them gives the increase that would be expected at a given age in the butter-fat percentage of one cow over that of another if at the certain age the first cow tested one percent over the second cow.

Of the information desired by the man who is to use these equations perhaps nothing is more important than a knowledge of what age a cow should make her record to give the best index of her potentialities in producing butter fat and the converse of this,—to what age should the records, made at different periods in the life of the cow, be corrected so that the maximum reliance may be placed on the corrected record as a true measure of the cow's ability. These questions can be easily answered by the equations themselves since the size of the coefficients of the b_s is a direct measure of the reliance which can be placed on a record made at a given year. By summing these coefficients for a given age and dividing by the total the average value may be obtained and the information desired obtained. As measured in this manner the age at which the butter-fat percentage determined for the milk most nearly represents the potentialities of the cow is the five-year lactation. The four-year, two-year and seven-year lactations are close behind the five-year with a difference so small as to be insignificant. The poorest year is three with ten close behind. The difference between the poorest (.4562) and the best (.5623) years is slight and very likely not significant. For the age to be predicted, too, the lactation at two years is best; the coefficient is .6076. The lowest coefficient is at 10 years of age (.4639). Here again the difference is not large and may not be significant.

THE CORRELATION OF THE BUTTER-FAT PERCENTAGE OF A LACTATION AT A GIVEN AGE WITH THE BUTTER-FAT PERCENTAGE OF TWO LACTATIONS

These constants are of special interest as measuring any difference in the contribution of the butter fat of one lactation as against that of another lactation. Further, these correlations, with the natural extension of them to cover the relation of the production of one lactation to the production of the cow's whole life, have the greatest appeal to the practical dairyman as he is primarily interested in the return the cow can

TABLE 38

Correlation surface for percentage of butter fat in the eight-months milk production at six years old and percentage of butter fat in the eight-months milk production at ten-and-above years old for pure-bred Jersey cows.

6 years old

10-and-above years old	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	6.20-6.40	Total
4.00-4.20	1										1
4.20-4.40	1	1		1						1	4
4.40-4.60	1										1
4.60-4.80		3		1			1				5
4.80-5.00			5	4							9
5.00-5.20		2		3	1	2	3		1		12
5.20-5.40		1	4		3	1	1				10
5.40-5.60		1	2				6		1		10
5.60-5.80		2	1							1	4
5.80-6.00							1				1
6.00-6.20		1								1	2
Total	3	11	12	9	4	3	12	0	2	3	59

TABLE 39

Correlation surface for percentage of butter fat in the eight-months milk production at seven years old and percentage of butter fat in the eight-months milk production at eight and nine years old for pure-bred Jersey cows.

7 years old

8 and 9 years old	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	Total
4.20-4.40	2				1	1	1					5
4.40-4.60	3		2	4	2	2		1				14
4.60-4.80			2	3	5	1	2					13
4.80-5.00				4	2	3	4	2	3			18
5.00-5.20				4	5	10	7	1	1	1		29
5.20-5.40		2	1	1	8	9	9	2		1		33
5.40-5.60					1	5	7	5		1	3	22
5.60-5.80					1		2	4	1	1		9
5.80-6.00							1	1	1	2		5
6.00-6.20											2	2
6.20-6.40											1	1
Total	5	2	5	16	25	31	33	16	6	6	6	151

TABLE 40

Correlation surface for percentage of butter fat in the eight-months milk production at seven years old and percentage of butter fat in the eight-months milk production at ten-and-above years old for pure-bred Jersey cows.

7 years old

10-and-above years old	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	Total
4.00-4.20		1								1
4.20-4.40	2	1	2						1	6
4.40-4.60		1								1
4.60-4.80	2	1			1					4
4.80-5.00		1		4	3	1				9
5.00-5.20	2	3	2	2	4	1		1		15
5.20-5.40	1		6		5	3	1			16
5.40-5.60			1	2	3	2		1	1	10
5.60-5.80		1	1	1			1		1	5
5.80-6.00							1			1
6.00-6.20		1		1					1	3
6.20-6.40									1	1
Total	7	10	12	10	16	7	3	2	5	72

TABLE 41

Correlation surface for percentage of butter fat in the eight-months milk production at eight and nine years old and percentage of butter fat in the eight-months milk production at ten-and-above years old for pure-bred Jersey cows.

8 and 9 years

10 years and older	3.80-4.00	4.00-4.20	4.20-4.40	4.40-4.60	4.60-4.80	4.80-5.00	5.00-5.20	5.20-5.40	5.40-5.60	5.60-5.80	5.80-6.00	6.00-6.20	Total
4.00-4.20				2									2
4.20-4.40			2		1	1		2	1				7
4.40-4.60			5	1									6
4.60-4.80				1	2	1		3					7
4.80-5.00	1		1		3		3	4	3				15
5.00-5.20			1	2	4	3		11	2	1	2		26
5.20-5.40			1	2	1	3	2	6	8	3	2		28
5.40-5.60				1	1	1	1	7	6	1	1		19
5.60-5.80						1	1		2	1	1	3	9
5.80-6.00													0
6.00-6.20						1			2			1	4
6.20-6.40												1	1
Total	1	0	10	9	12	11	7	33	24	6	6	5	124

be expected to make during her lifetime. These two problems have been approached in two ways; (1) the correlation of the butter-fat percentage of a given year with the butter-fat percentage of the milk of the two-year period using the data presented in tables 7 to 41; (2) a special set of correlations for the correlation of the mean butter-fat percentage of five lactations with the butter-fat percentage for each lactation.

The first set of correlations are easily determined where the means and standard deviations are known and $x = y + z$, from the relations.

$$\bar{x} = \bar{y} + \bar{z} \qquad \sigma_x = \sqrt{\sigma_y^2 + \sigma_z^2 + 2\, r_{yz}\, \sigma_y\, \sigma_z}$$

The product moments are (Harris 1918)

$$\Sigma\,(xy) = \Sigma\,(y^2) + \Sigma\,(yz)$$
$$\Sigma\,(xz) = \Sigma\,(z^2) + \Sigma\,(yz)$$

These correlations when so determined are given in table 42.

These correlations are all high as correlations on this kind of material go. They range in value from 0.9181 ± .0090 to 0.7048 ± .0343. The value of the lowest does not really give a true indication of the range for the next lowest observation is .7872 ± .0334. The correlations and in fact the constants for this distribution of butter-fat percentage are somewhat abnormal as compared with the constants for the other distributions of seven years, as has been previously noted. No reason for this is known. In any case the correlations are high and point to a close agreement between the butter-fat percentage in the milk of one lactation with that of the given year plus the record of another lactation at some other age.

The difference column is of a good deal of interest as it demonstrates any difference in the relative weight which should be given one lactation over that of another lactation as a measure of the lactation's merits as an indication of the cow's future production or value as a producer of butter fat. Barring the abnormally high difference for three and seven years the differences are all small in comparison with their probable errors. No consistency in the sign of these differences can be noted. It is altogether probable from these results that no real difference exists in the relative production of the different lactations of a cow's life. It seems, from this, that environmental differences bring about the variations in these correlations.

Of more importance than the comparison of the lactation sum with that of each component is the comparison of the mean butter-fat percentage over a number of lactations with those of the mean butter-fat percentages of the individual lactations which contribute to the mean

Correlation between the butter-fat utter-fat percentage of a lactation rec- o year.

Character*	tion	Difference	$\frac{\text{Difference}}{E\ \text{Difference}}$
2 and (2 + 3)	0.8 .0114	— .0142 ± .0170	.83
2 and (2 + 4)	.8 .0118	— .0195 ± .0181	1.08
2 and (2 + 5)	.9 .0132	.0211 ± .0172	1.23
2 and (2 + 6)	.9 .0141	.0244 ± .0182	1.34
2 and (2 + 7)	.9 .0165	.0371 ± .0203	1.83
2 nad (2 + 8 and 9)	.8 .0167	.0280 ± .0213	1.31
2 and (2 + 10 and older)	.8 .0261	.0103 ± .0354	.29
3 and (3 + 4)	.9 .0116	.0137 ± .0154	.89
3 and (3 + 5)	.9 .0136	.0278 ± .0173	1.61
3 and (3 + 6)	.8 .0142	.0133 ± .0190	.70
3 and (3 + 7)	.8 .0343	.1567 ± .0385	4.07
3 and (3 + 8 and 9)	.8 .0211	.0438 ± .0267	1.64
3 and (3 + 10 and older)	. .0390	.0158 ± .0521	3.03
4 and (4 + 5)	. .0095	.0042 ± .0131	.32
4 and (4 + 6)	. .0151	.0006 ± .0214	.03
4 and (4 + 7)	. .0136	— .0122 ± .0202	.60
4 and (4 + 8 and 9)	. .0165	— .0139 ± .0244	.57
4 and (4 + 10 and older)	.8 .0209	— .0177 ± .0315	.56
5 and (5 + 6)	.8 .0142	.0140 ± .0190	.74
5 and (5 + 7)	.8 .0167	.0093 ± .0229	.41
5 and (5 + 8 and 9)	.8 .0120	— .0030 ± .0172	.17
5 and (5 + 10 and older)	.8 .0235	— .0396 ± .0377	1.05
6 and (6 + 7)	.8 .0148	.0246 ± .0192	1.28
6 and (6 + 8 and 9)	.8 .0154	.0137 ± .0210	.65
6 and (6 + 10 and older)	.8 .0334	.0488 ± .0426	1.15
7 and (7 + 8 and 9)	.8 .0112	.0076 ± .0154	.49
7 and (7 + 10 and older)	.8 .0222	— .0171 ± .0330	.52
8 and 9 and (8 and 9 + 10 and older)	.8 .0160	.0325 ± .0203	1.60

* Under characters are recorded fir *and* signifies that this lactation record for butter-fat percentage was correl cords made at the ages indicated in the parenthesis. The *age* at which the fi nthesis. The plus signifies this record was added to the record made at th

butter-fat percentage of the longer period. This point is taken up in the next section.

CORRELATION BETWEEN THE MEAN BUTTER-FAT PERCENTAGE OF THE FIRST FIVE LACTATIONS AND THE MEAN BUTTER-FAT PERCENTAGE OF THE INDIVIDUAL LACTATIONS

Of perhaps even more interest physiologically and practically are the correlations of the butter-fat percentage of one lactation with the butter-fat percentage as determined for a number of lactations. For this purpose certain of the records on which the correlations of table 30 were based, were chosen for this purpose. These records included the first five lactations of the cow's life. The correlations and other constants for these collected from tables 44 to 48 are given in table 43.

TABLE 43

Correlation coefficients and constants for butter-fat percentage over five lactations and the butter-fat percentage for the individual lactations.

Age when lactation commenced	Mean butter-fat percentage	Standard deviation for butter-fat percentage	Coefficient of variation of the butter-fat percentage
2 years to 3 years....	5.245 ± .035	0.491 ± .025	9.35 ± .46
3 years to 4 years....	5.227 ± .035	.485 ± .025	9.29 ± .46
4 years to 5 years....	5.291 ± .036	.502 ± .026	9.48 ± .46
5 years to 6 years....	5.225 ± .033	.462 ± .023	8.83 ± .46
6 years to 7 years....	5.177 ± .031	.425 ± .022	8.20 ± .41
Five-lactation butter-fat percentage...	5.216 ± .029	.399 ± .020	7.651 ± .39

TABLE 43 (continued)

Correlation coefficients and constants for butter-fat percentage over five lactations and the butter-fat percentage for the individual lactations.

Age when lactation commenced	Correlation of individual lactation records and the record for the five lactations	Correlation ratios for individual records and the record of the five lactations	Regression equations for the five-lactation butter-fat percentage as calculated from any given lactation
2 years to 3 years....	+ 0.797 ± .026	0.827 ± .023	$B_T = 1.819 + .648b_2$
3 years to 4 years....	.836 ± .022	.855 ± .019	$B_T = 1.621 + .688b_3$
4 years to 5 years....	.862 ± .018	.876 ± .017	$B_T = 1.591 + .685b_4$
5 years to 6 years....	.857 ± .019	.873 ± .017	$B_T = 1.349 + .740b_5$
6 years to 7 years....	.784 ± .028	.815 ± .024	$B_T = 1.406 + .736b_6$

Table 43 shows that the standard deviation of the butter-fat percentage for the mean of the five lactations in these 88 cows is lower than the standard deviation of these cows for any lactation. The coefficient of variation for the five-lactation average butter-fat percentage is consequently lower than the coefficient of variation for the individual lactations. The mean coefficient of variation for the individual lactations is 9.03. This mean value is 1.38 greater than is the coefficient of variation for the five-lactation butter-fat percentage. This difference appears to be slightly significant indicating a less variability for the butter-fat percentage over long periods than over a period so short as one lactation.

The correlation coefficients for the relation of the individual lactation's butter-fat percentage for the five lactations are all high correlations as the run of correlations for this kind of data go. Compared with the similar data on milk production the average correlations for milk production are + 0.818 and for butter-fat percentage + 0.827. The value of the correlation coefficients is so high in each case that the average milk production or butter-fat percentage over a number of lactations can be predicted quite accurately from the productions obtained for any lactation.

If it is admitted that there is a regulatory mechanism controlling the amount of milk produced by a cow in any lactation as it seems that it must be admitted from the evidence, then the large size of the correlations indicates clearly that this mechanism is working quite accurately in governing the relative amount of milk a cow will produce from lactation to lactation.

The precision of action of this mechanism for the secretion of butter fat in a given cow's milk is greater than is the precision of action of the ovary of a hen in secretion of eggs as may be seen from the data of HARRIS and BLAKESLEE (1918). For the White Leghorn pullets the correlation of their monthly productions with their annual production ranges from + 0.373 ± .030 to 0.695 ± .018. The range of the correlations for butter-fat percentage is 0.784 ± .028 to 0.862 ± .018. The mean correlation coefficients stand in the relation 0.556 to .827 or 1 to 1.49.

The correlation coefficients measure the amount of association between the butter-fat percentage for one year and that for the five-lactation total quite accurately as may be seen by a comparison of their values with those of the correlation ratios. The difference of these values is in all cases very small in comparison with its probable error.

The work of this STATION has often shown the desirability of having

TABLE 44

Correlation surface for butter-fat percentage of eight-months milk production for the first five lactations in the cow's life and butter-fat percentage of the eight-months milk production at 3 years old for pure-bred Jersey cattle.

Average butter-fat percentage for first five lactations

2 years old	4.40-4.50	4.50-4.60	4.60-4.70	4.70-4.80	4.80-4.90	4.90 5.00	5.00-5.10	5.10-5.20	5.20-5.30	5.30-5.40	5.40-5.50	5.50-5.60	5.60-5.70	5.70-5.80	5.80-5.90	5.90-6.00	6.00-6.10	6.10-6.20	6.20-6.30	Total
4.00-4.20			1																	1
4.20-4.40		1	1																	2
4.40-4.60		1	3	2	1		2													9
4.60-4.80	1		2	1	1		1						1							7
4.80-5.00		1		1	2	2	1	1			1									9
5.00-5.20			1		3	1	1	1	1											8
5.20-5.40					1		1	2	1	4	3		2							14
5.40-5.60							1	2	2	6	3	3	1					1		19
5.60-5.80									1	2	2	2								7
5.80-6.00										1	2		1		1		1		1	7
6.00-6.20											1		2	1						4
6.20-6.40																			1	1
Total	1	3	8	4	8	3	7	6	5	13	12	5	7	1	1	0	1	1	2	88

TABLE 45

Correlation surface for butter-fat percentage of eight-months milk production for the first five lactations in the cow's life and the butter-fat percentage of the eight-months milk production at 3 years old for pure-bred Jersey cattle.

Average butter-fat percentage for first five lactations

3 years old	4.40-4.50	4.50-4.60	4.60-4.70	4.70 4.80	4.80-4.90	4.90-5.00	5.00-5.10	5.10-5.20	5.20-5.30	5.30-5.40	5.40-5.50	5.50-5.60	5.60-5.70	5.70-5.80	5.80-5.90	5.90-6.00	6.00-6.10	6.10-6.20	6.20-6.30	Total
4.20-4.40	1	1	1																	3
4.40-4.60			4	3																7
4.60-4.80		1	3		4		1													9
4.80-5.00				1	1	1	4	1	1				1							10
5.00-5.20					2	2	1	3	1	4	1									14
5.20-5.40					1		1	2	1	3	3			1						12
5.40-5.60									1	4	3	1	2							11
5.60-5.80		1							1	1	5	2	2					1		13
5.80-6.00												2	2							4
6.00-6.20										1					1		1			3
6.20-6.40																			1	1
6.40-6.60																			1	1
Total	1	3	8	4	8	3	7	6	5	13	12	5	7	1	1	0	1	1	2	88

a means of predicting a result from a known variable. The regression equations given in the last column of table 43 give a means for doing this for the butter-fat percentage over the first five lactations of a cow's life from the butter-fat percentage over any lactation. The equation for the first lactation will be used in a later section of this paper for a table of practical interest.

THE INFLUENCE OF ENVIRONMENTAL CHANGES ON THE BUTTER-FAT PERCENTAGE FROM LACTATION TO LACTATION, QUANTITATIVELY CONSIDERED

As discussed in the introduction the author takes it to be one of the final objects of investigations in genetics to determine the relative importance of environment and heredity in the final appearance of the characters observed. The importance of this knowledge in the present-day studies of inheritance has often been overlooked in the fact that the characters dealt with have little relative variability with little or no overlap. The increasing desirability of a knowledge of inheritance of economic characters has forced home as perhaps nothing else could the necessity of knowing the extent to which the stresses and strain of environment influence the expression of the gene.

For the character which we are studying, the most favorable environ-

TABLE 46

Correlation surface for butter-fat percentage of eight-months milk production for the first five lactations in the cow's life and the butter-fat percentage of the eight-months milk production at 4 years old for pure-bred Jersey cattle.

4 years old	Average butter-fat percentage for first five lactations																			
	4.40-4.50	4.50-4.60	4.60-4.70	4.70-4.80	4.80-4.90	4.90-5.00	5.00-5.10	5.10-5.20	5.20-5.30	5.30-5.40	5.40-5.50	5.50-5.60	5.60-5.70	5.70-5.80	5.80-5.90	5.90-6.00	6.00-6.10	6.10-6.20	6.20-6.30	Total
4.20-4.40	1		1																	2
4.40-4.60		1	2																	3
4.60-4.80		2	3		3		1	1												10
4.80-5.00			2	1	4	1	2		1											11
5.00-5.20				1	1		1	1	4	2										10
5.20-5.40				2		1	2	3		7	3	2								20
5.40-5.60						1	1	1		2	6	1								12
5.60-5.80										1	3	1	3							8
5.80-6.00												1	2	1	1					5
6.00-6.20													1						1	2
6.20-6.40										1			1				1			3
6.40-6.60																				0
6.60-6.80																			1	1
6.80-7.00																		1		1
Total	1	3	8	4	8	3	7	6	5	13	12	5	7	1	1	0	1	1	2	88

TABLE 47

Correlation surface for butter-fat percentage of eight-months milk production for the first five lactations in the cow's life and the butter-fat percentage of the eight-months milk production at 5 years old for pure-bred Jersey cattle.

Average butter-fat percentage for first five lactations

5 years old	4.40-4.50	4.50-4.60	4.60-4.70	4.70-4.80	4.80-4.90	4.90-5.00	5.00-5.10	5.10-5.20	5.20-5.30	5.30-5.40	5.40-5.50	5.50-5.60	5.60-5.70	5.70-5.80	5.80-5.90	5.90-6.00	6.00-6.10	6.10-6.20	6.20-6.30	Total
4.00-4.20		1																		1
4.20-4.40				1																1
4.40-4.60	1	1	2	1	1															6
4.60-4.80		1	3	1	2	1	1													9
4.80-5.00			2		5		2													9
5.00-5.20						2	3	5	4	1	1		1							17
5.20-5.40			1	1			1		1	4	3	2								13
5.40-5.60								1		6	6	1	2		1					17
5.60-5.80										1		2	3							6
5.80-6.00											1		1	1			1			4
6.00-6.20										1	1								2	4
6.20-6.40																				0
6.40-6.60																				0
6.60-6.80																		1		1
Total	1	3	8	4	8	3	7	6	5	13	12	5	7	1	1	0	1	1	2	88

TABLE 48

Correlation surface for butter-fat percentage of eight-months milk production for the first five lactations in the cow's life and the butter-fat percentage of the eight-months milk production at 6 years old for pure-bred Jersey cattle.

Average butter-fat percentage for first five lactations

6 years old	4.40-4.50	4.50-4.60	4.60-4.70	4.70-4.80	4.80-4.90	4.90-5.00	5.00-5.10	5.10-5.20	5.20-5.30	5.30-5.40	5.40-5.50	5.50-5.60	5.60-5.70	5.70-5.80	5.80-5.90	5.90-6.00	6.00-6.10	6.10-6.20	6.20-6.30	Total
4.00-4.20			1																	1
4.20-4.40			1	1																2
4.40-4.60	1	2	1																	4
4.60-4.80			2	1	4	1	2			1										11
4.80-5.00				2	3			2		2	3									12
5.00-5.20			3			1	2	3	2	1	2									14
5.20-5.40		1			1	1	1	1	2	5	3		1							16
5.40-5.60							2		1	3	3	3	4	1						17
5.60-5.80										1		1	1					1		4
5.80-6.00											1	1	1		1		1			5
6.00-6.20																			1	1
6.20-6.40																			1	1
Total	1	3	8	4	8	3	7	6	5	13	12	5	7	1	1	0	1	1	2	88

ment may be considered to bring about the full expression of the hereditary complex. Taking this definition it follows that environmental changes, however slight they may be, are detrimental to the full expression of the mammary gland's possibilities. Since this study deals with the records of the same individual cow for successive lactations the inherited complex for her potential butter-fat percentage may be said to be given and constant.

Some measure of the influence of environmental changes from year to year may be gained by a study of the coefficients of correlation for the butter-fat percentage of one lactation with that of another lactation and the coefficient of correlation between the butter-fat percentage of one lactation and the butter-fat percentage over a number of lactations. In the first case the environmental changes are acting on both lactations whereas in the second case the environmental change only acts on one lactation as the mean environment will be quite constant over the longer period. This measure is not altogether satisfactory. It is however perhaps the best measure that it is possible to obtain under the difficulty of working individually with the greater number of variables which make up environment. The data used for this comparison are the same as those on which the correlations of table 43 were based. The correlations were first determined for the given lactation correlated with the mean butter-fat percentage of the first four of the first five lactations which did not include the given lactation. These data are given in table 49.

TABLE 49

Lactations correlated	Correlation coefficients	Average correlation coefficients		Correlation coefficients for the given lactation correlated with the mean butter-fat percentage of first four non-inclusive lactations	
1 and 2	0.711 ± .036	1st lactation		1st and	
1 and 3	.588 ± .047	and 2nd, 3rd, 4th, 5th	0.611 ± .044	2+3+4+5	0.690 ± .038
1 and 4	.667 ± .040	2nd lactation		2nd and	
1 and 5	.480 ± .055	and 1st, 3rd, 4th, 5th	.664 ± .040	1+3+4+5	.775 ± .029
2 and 3	.687 ± .038	3rd lactation		3rd and	
2 and 4	.646 ± .042	and 1st, 2nd, 4th, 5th	.658 ± .041	1+2+4+5	.778 ± .028
2 and 5	.611 ± .045	4th lactation		4th and	
3 and 4	.699 ± .037	and 1st, 2nd, 3rd, 5th	.647 ± .042	1+2+3+5	.751 ± .031
3 and 5	.658 ± .041	5th lactation		5th and	
4 and 5	.576 ± .048	and 1st, 2nd, 3rd, 4th	.581 ± .047	1+2+3+4	.667 ± .040

The average correlation coefficients of a given lactation in comparison with the other four of the series are in general 0.1 lower than are the correlation coefficients of the given lactation with the average butter-fat percentage of the other four lactations. This difference is consistent in amount and in sign. This consistency rather than the amount of the difference leads to the conclusion that these slight differences are biologically significant. The environmental changes may be said to lower the coefficient of correlation about one-seventh of its value. This is of course for an average environment. The lowering of the correlation coefficient from the best environmental complex, for the full expression of the cow's hereditary ability to produce high concentration of butter-fat in her milk, would of course be greater than this by a small amount. These results are very similar to those of the previous study on milk quantity, the two sets of correlation coefficients differing by about the same amount.

PRACTICAL ASPECTS OF THE CORRELATIONS FOR BUTTER-FAT PERCENTAGE OF ONE LACTATION WITH THE BUTTER-FAT PERCENTAGE OF ANOTHER LACTATION

As many of these results have a highly practical bearing it may be well to illustrate one of the uses to which they may be put. The question of what animals shall be saved for milk production and the perpetuation of the herd is a constantly recurring one in dairy practice. The correlations just deduced in table 43 show that the basis of this selection should be the records of the previous lactation. Suppose the herd is composed of 1000 cows which have just completed their first lactation. The equations derived from table 4 on page 263 allows the determination of the distribution of the cows with respect to their butter-fat percentage providing they are from a similar population to these pure-bred Jersey cattle. The equation for this curve,

$$y = 17.8901 \left(1 + \frac{x^2}{3.9271}\right)^{-9.6974} e^{5.2895 \tan^{-1} \frac{x}{1.9817}},$$

allows the calculation of the distribution of these one-thousand cows as shown in the second column of table 50. From the eight-months butter-fat percentage table 43 gives the equation to determine the expected mean butter-fat percentage for the first five lactations. The equation is

$$B_T = 1.819 + .648b_2$$

where B_T is equal to the butter-fat percentage of the first five lactations.

The data may be tabled for most easy reference by summation of the number of cows from both ends of the distribution and tabling the mean butter-fat percentage of this group. This has been done for table 50.

TABLE 50

Actual butter-fat percentage of one-thousand two-year-old cows and the expected five-lactation butter-fat percentage for any division of that herd or per cow for any division of it.

Actual butter-fat percentage	Number of cows with given butter-fat percentage	Expected butter-fat percentage for the first five lactations	Number of cows summed from lowest to highest butter-fat percentage	Number of cows summed from highest to lowest butter-fat percentage	Expected average butter-fat percentage, lowest to highest	Expected average butter-fat percentage highest to lowest
3.75-3.85	1	4.281	1	1000	4.28	5.23
3.85-3.95	2	4.346	2	999	4.32	5.23
3.95-4.05	3	4.411	5	998	4.37	5.23
4.05-4.15	5	4.476	10	995	4.42	5.23
4.15-4.25	7	4.541	17	990	4.47	5.24
4.25-4.35	11	4.605	28	983	4.53	5.24
4.35-4.45	17	4.670	45	972	4.58	5.25
4.45-4.55	25	4.735	70	955	4.64	5.26
4.55-4.65	34	4.800	104	930	4.69	5.27
4.65-4.75	45	4.865	149	896	4.74	5.29
4.75-4.85	56	4.929	205	851	4.79	5.31
4.85-4.95	66	4.994	271	795	4.84	5.34
4.95-5.05	75	5.059	345	729	4.89	5.37
5.05-5.15	80	5.124	426	655	4.93	5.41
5.15-5.25	82	5.189	508	574	4.97	5.45
5.25-5.35	80	5.253	588	492	5.01	5.49
5.35-5.45	75	5.318	664	412	5.05	5.54
5.45-5.55	68	5.383	731	336	5.08	5.59
5.55-5.65	59	5.448	790	269	5.11	5.64
5.65-5.75	49	5.513	839	210	5.13	5.69
5.75-5.85	40	5.577	880	161	5.15	5.74
5.85-5.95	32	5.642	911	120	5.17	5.80
5.95-6.05	24	5.707	936	89	5.18	5.86
6.05-6.15	18	5.772	954	64	5.19	5.91
6.15-6.25	14	5.837	968	46	5.20	5.97
6.25-6.35	10	5.901	978	32	5.21	6.02
6.35-6.45	7	5.966	985	22	5.22	6.08
6.45-6.55	5	6.031	990	15	5.22	6.13
6.55-6.65	4	6.096	994	10	5.22	6.18
6.65-6.75	3	6.161	996	6	5.22	6.23
6.75-6.85	2	6.225	998	4	5.23	6.27
6.85-6.95	1	6.290	999	2	5.23	6.32
6.95-7.05	1	6.355	1000	1	5.23	6.35

The first column of the table gives the butter-fat test of the milk produced in the first eight months of lactation for two-year-old cows. The second column gives the distribution of a herd of one-thousand two-year-old cows of like constitution to the pure-bred Jersey cattle which are being studied. All the calculations have been carried to several decimal places beyond the tabled constants as only by so doing would the results have been more than approximately correct. The third column gives the butter-fat percentage to be expected for the first-five-lactations milk of cows which have the butter-fat percentage of those shown in the first column.

Noting the expected butter-fat percentage of the highest and lowest tested cows in this column it is seen that those cows which test very low in butter-fat (3.75 to 3.85) in their first lactation on the average increase 0.581 percent of butter-fat for the milk of their first five lactations; for the highest butter-fat-test cow in their two-year lactations the butter-fat percentage for their first five lactations is on the average 0.645 percent of butter-fat lower than is the test for the first lactation test.

The third and fourth columns give the summations of the number of cows from the lowest butter-fat test to the highest and from the highest butter-fat test to the lowest. The last two give the butter-fat test expected for these one-thousand two-year-old Jersey heifers when the herd is divided at any given place. To illustrate suppose the owner is to cull this herd so that only cows which produced 5.25 percent of butter fat in their first lactation will remain in it, what percent of butter fat could he expect the remainder of the herd to produce? The answer is found to be 5.49 percent in the last column of the table on the line with the 5.25-5.35 butter-fat percentage of the first column. Should it have been desirable to know the butter-fat percentage of the culled section of the herd this is found in the sixth column to be 4.97 on the line with 5.15-5.25 of the first column. The number of individuals remaining in this herd after culling may be seen in the fourth and fifth columns. Since these are tabled for 1000 individuals they may be easily reduced to percentages should it be convenient to deal with the results in this way.

These results are of course only applicable to herds similar to the one being studied. The comparison of the butter-fat test makes it seem likely that with only small error these results may be used for the whole of the Jersey breed and possibly the Guernsey breed.

THE RELATIVE CONTRIBUTION OF ONE LACTATION'S BUTTER-FAT PERCENTAGE TO THE BUTTER-FAT PERCENTAGE OVER THE FIRST FIVE LACTATIONS

The correlation between the five-lactations butter-fat percentage and deviation of the single component lactation from its probable value measures a relation of considerable significance. This significance is fairly clear, for should such a correlation exist it proves a difference in the lactation values that concern the butter-fat percentage of a cow. Such a difference would indicate a distinct set of factors influencing the butter-fat percentage at one lactation but not so influencing the others. Such a correlation has been shown for egg production in the Barred Plymouth Rocks (PEARL and SURFACE 1911) and in the White Leghorn pullets (HARRIS and BLAKESLEE 1918). Table 51 gives the correlation coefficients for this relationship.

TABLE 51

Correlation between the butter-fat percentage of the first five lactations and the deviation of the individual records from their probable values.

Variable correlated	Correlation coefficient	Correlation coefficient
		Probable error
2-year butter-fat percentage and 5-lactation butter-fat percentage.........	— 0.035 ± .072	.482
3-year butter-fat percentage and 5-lactation butter-fat percentage.........	0.023 ± .072	.382
4-year butter-fat percentage and 5-lactation butter-fat percentage.........	0.108 ± .071	1.52
5-year butter-fat percentage and 5-lactation butter-fat percentage.........	— 0.018 ± .072	.25
6-year butter-fat percentage and 5-lactation butter-fat percentage.........	— 0.233 ± .068	3.43

The correlation coefficients of table 51 are plus, twice, and minus, three times. They range in value from + 0.108 to — 0.233, from 0.25 to 3.43 times their probable error. Only one correlation coefficient can be considered significant as judged by this standard. Such small correlations with no consistency in sign make it doubtful if the butter-fat percentage of any lactation is anything but random so far as the relative contributions of the high and low butter-fat producers to the life-time butter-fat percentage of cows' milk is concerned. These facts make it clear that no difference in the relative concentration of butter-fat or separate cycles of this butter-fat exist. The factors governing the secretion of milk fat can, in view of this conclusion, be said to control the butter-fat secretion throughout the cow's life and not for any period as short as one lactation.

SUMMARY

This paper has as its object the presentation of quantitative data along with the biometrical analysis of the same, on the normal variations and correlations of the butter-fat percentage contained in Jersey milk during the different lactations of the cow's life. In this sense this paper is the copartner to the previous paper in this series (GOWEN 1920) dealing with the variations of milk yield for these same Jersey cows. The data used for this study are especially suited to the objects of this investigation. They are from a strictly homogeneous group of records for the butter-fat percentage of normal, healthy, pure-bred Jersey cows maintained under the conditions of a large farm, managed by a well-trained man thoroughly versed in his business. While stated in general form the conclusions are not presumed to be more general than the data on which they are based. Certain comparisons with other data contained in the body of the paper make it seem altogether probable that to a large degree the conclusions are of general application to most of the other dairy breeds of cattle. The well recognized need for such data and analysis in physiological and genetic research and the significant relation such data have to the improvement in yield and in the selection of cows to remain within the herd have made it seem desirable to present as complete numerical data as possible.

The following paragraphs briefly state the conclusions which are considered of most importance. Unless stated to the contrary, these conclusions apply to the pure-bred Jersey herd the data for which are given in this paper.

This investigation deals with 1713 records for the butter-fat percentage of the first eight-months lactation, each cow milking at least nine months. The frequency polygons for butter-fat percentage are nearly symmetrical. One only, at the ages nine to ten years, diverges noticeably from this form. The range of variation extends from 3.65 to 6.95 percent of butter fat. The height of the modal ordinate and

the shape of the polygons do not change markedly with age as was the case for the curves for milk production of the same data. Only one mode is evident in any of the histograms.

Comparatively studied, the butter fat in the milk secreted by twenty-eight breeds of cows shows that the means of these breeds form a distribution distinctly bimodal in character. The mode of the first curve coming at about 3.7 percent and of the second at about 5.0 percent of butter fat.

Study of the standard deviation of typical members of the above groups shows a greater scatter in the butter-fat percentages in the group where the butter-fat concentration is high than in the group where the butter-fat concentration is low.

The analytical constants show that the butter-fat-percentage histograms of the eight-months milk production are PEARSON's type I, III, IV and V, and normal curves. The skewness of these curves, where they are skew, is plus, four times, and minus, twice. The skewness for each curve is small in amount. This relatively small size of the skewness has some interest to those investigators who deal with advanced-registry records, since within a small error normal curves may be considered to describe the general population from which the truncated portion is drawn in advanced-registry selection.

The correlation coefficient of butter-fat percentage with age is 0.1126 ±.0161. The correlation ratio calculated from the same data is 0.1478± .0159. The excellent agreement between these two constants shows that the regression of butter-fat percentage on age is to all intents and purposes linear. The highest mean butter-fat percentage occurs in the first lactation of a cow's life. From this high point there is a slight decline in the butter-fat percentage as age advances.

Comparison of these correlations with those for the other breeds, Guernsey, Ayrshire and Holstein-Friesian, leads to the following law expressing the relation between age and butter-fat percentage: Each increment of time added to a cow's life causes a slight decline in the relative amount of butter-fat which that cow can secrete into her milk

The standard deviations of butter-fat percentage decreases very slightly with advancing years. The function describing this decrease is a linear one.

Correlation tables for butter-fat percentage for lactations during certain age groups are presented.

The mean butter-fat percentages derived from these tables remain, within the limits of random sampling, the same for any given age, at test, irrespective of the age at which the same cows were subsequently tested.

The standard deviations of the butter-fat percentage for the various age groups remained at the same value within the limits of sampling irrespective of the age at which a subsequent test was made.

The coefficients of variation for the butter-fat percentage of the various ages also remained approximately constant irrespective of the age at which a subsequent test was made on the same cows.

These facts show that cows kept in the herd at any given time were all equally likely to be kept in the herd at any other time so far as their eight-months butter-fat percentage was concerned. This point removes any possible criticism of the conclusions drawn from these studies on the ground that the data were subject to selection.

The correlation coefficients for the butter-fat percentage of one lactation with the butter-fat percentage of another lactation range from 0.2470 ± .0640 to + 0.6781 ± .0310. The signs of these correlation coefficients are all plus. The graphs of these correlations are approximately linear. Very little or no difference occurs in the values of the correlation coefficients of the butter-fat percentage at a given age with the butter-fat percentage for another age.

Comparison of these correlation coefficients with those for milk production shows that the average coefficient of correlation for the butter-fat percentage of one lactation with that of another lactation is + 0.5215, and the average correlation coefficient for milk production of one lactation with another lactation is + 0.5352. The correlations for milk production are higher, although not significantly so, than those for butter-fat percentage. Such being the case it follows that the relative accuracy in the use of one lactation record to predict the expected record of another lactation is approximately the same for the butter-fat percentage and for milk yield.

These correlation coefficients are high for data of economic importance. The mean coefficient of correlation for monthly egg production with the yearly egg production is + 0.446. The range of these correlation coefficients is also lower than the correlation coefficients for butter-fat percentage. In terms of physiology this difference in the correlation coefficients for ovulation and butter-fat percentage means that the mechanism controlling the mammary gland has greater precision in its action than has the mechanism controlling the action of the ovary.

The correlation ratios were calculated. Comparison of these correlation ratios with the correlation coefficient shows all of the regressions to be linear regressions.

The means, standard deviations and correlation coefficients give the necessary constants to form the straight-line equations for the prediction of the butter-fat percentage of any given lactation at any age from these known variables at another age. These equations are given in table 37. Examination of these equations shows that the age at which the butter-fat percentage determined for the milk most nearly represents the cow's potentialities is five years. The age at which to predict the butter-fat percentage of the other years, to arrive at the most nearly accurate result, is two years. The correlation coefficients for butter-fat percentage of a given lactation with a butter-fat percentage of two lactations of which the given lactation is one component, range from 0.7048 ± .0343 to 0.9181 ± .0090.

The correlation coefficients of the butter-fat percentage of one lactation with the butter-fat percentage of the first five lactations range from 0.784 to 0.862. Each regression is shown to be linear by a comparison of the correlation coefficients and correlation ratios. The regression equations for the prediction of the five-year butter-fat percentage from the percentage of any given year are given in table 43.

The difference of the correlation coefficients for one lactation's butter-fat percentage with that of another lactation and for one lactation's butter-fat percentage with the butter-fat percentage over four lactations has been determined. The difference of these correlation coefficients gives a means of measuring the effect of environmental changes on the butter-fat percentage of one lactation, since in the first case the environmental changes of the different lactations as they affect milk production make themselves felt in the correlation coefficients, and in the second case, the use of the butter-fat percentage for the four lactations give a mean of these environmental effects. The result of this comparison showed that environmental changes lower the correlation coefficients from one-seventh to one-eighth of what it would be under the conditions of an average environment. The environmental effect on butter-fat percentage is consequently about the same as the environmental effect on milk production. Since the internal governing action of the cow for butter-fat percentage has nearly six times the effect in controlling butter-fat percentage that the environmental changes have, it follows that this internal mechanism (probably hereditary) plays a greater part in determining what these Jerseys' butter-fat percentage will be than does the environment.

The equations to predict the butter-fat percentage of the first five lactations from the butter-fat percentage of a single lactation are of great importance in culling mediocre cows from the herd. The use of these equations is illustrated in table 50.

The relative contribution of the butter-fat percentage of the different lactations to butter-fat percentage over the first five lactations is the same up to the fifth lactation. In the fifth lactation the correlation coefficients would seem to indicate a slightly less relative contribution of the higher-test cows to the five-lactation butter-fat percentage than is the relative contribution of the lower-test cows. In general, since the significance of this correlation is only slight, the conclusion can be safely drawn, that the factors which govern butter-fat percentage have their regulatory power maintained in the same relative strength throughout the life of the cow to the exclusion of any group of factors acting within this life for short periods.

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LINKAGE IN MICE AND RATS*

L. C. DUNN
Storrs Agricultural Experiment Station, Storrs, Connecticut

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INTRODUCTION

The important generalizations concerned with the mechanism of heredity have until recently been supported almost wholly by evidence drawn from breeding experiments with insects. The reasons for this are obvious, for the proof and application of the chromosome theory of heredity depends primarily on linkage. The study of linkage can only be prosecuted where animals or plants exhibiting well defined unit variations can be bred in large numbers. Critical evidence must hence be accumulated by strictly experimental methods. These conditions were at first satisfied only by the vinegar-fly, *Drosophila melanogaster,* which still remains in many ways the most ideal material for experimentation in heredity. However, generalizations so important as to affect considerably our ideas of evolution, should be tested for other organisms, and the last few years have witnessed an extension and confirmation of the chromosome theory by investigators working with many kinds of animals and plants.

*The studies on which this paper is based were made at the Bussey Institution, HARVARD UNIVERSITY.

Most recently, perhaps, the mammals have been added to the list of animals exhibiting linkage. Investigation in the higher animals must of necessity proceed more slowly than in either plants or insects. The mammals as compared with the insects or plants, are slow breeders. Their period of gestation is relatively long, the numbers born in each litter are small, and the difficulties regarding space, maintenance and disease are many. The number of known variations is small, and since most of the species available for laboratory study are characterized by many chromosomes, the probability of more than one character being located in each chromosome is small.

Nevertheless, it is important that search should be made in the mammals for data bearing on the chromosome theory. Critical evidence is that derived from the study of the linkage relations between genes, and the establishment of linkage has as its object the localization of the hereditary determiners. Some evidence of the localization of genes in mammals is now available. That linkage has not often been found is a not unexpected or entirely negative result, for it may often be shown that the genes for certain characters are not linked (independent). This fact localizes the genes in different chromosomes, which is in many ways as definite a result as the establishment of linkage which localizes them in the same chromosome.

In the paragraphs which follow there are presented the results of experimental studies of two cases of linkage in rodents, one in some detail. Later it is planned to summarize the results of investigations of heredity in mice, which indicate that other genes for color variations are not linked but independent.

ESTABLISHED CASES OF LINKAGE IN MICE AND RATS

Previous investigations

There are now four well established cases of linked genes in rats and mice, and these are also the only known cases of linkage (aside from sex-linkage) in mammals. CASTLE and WRIGHT (1915) reported the first of these cases. They found evidence of linkage between the genes for two new color variations of the Norway rat, red-eyed yellow and pink-eyed yellow, which had been described previously by CASTLE (1914). Subsequently CASTLE (1916, 1919) investigated this linkage in greater detail and found the crossover value between these two genes to be about 18.3 percent. This value was calculated from a total of 4476 young and probably describes quite accurately the relation of the gene for red-eye to that for pink-eye. Crossing over in both sexes was indicated.

The publication of the first report on this linkage was soon followed by a preliminary report by HALDANE, SPRUNT and HALDANE (1915) on the linkage between the genes for pink-eye and albinism in house mice. The authors preferred, however, to explain the results of their experiments in terms of the reduplication hypothesis. They found the gametic series 1:3:3:1 to be characteristic of the relation between these two genes, as compared with the gametic series 1:1:1:1, normally expected from hybrids between two independently segregating characters.

Data for this cross had been extant since the work of DARBISHIRE (1904) and of CUÉNOT (1907). Both of these authors had crossed pink-eyed mice with albinos. Their data showed that the two characters were distinct, for in the first generation dark-eyed, full-colored mice were produced. DARBISHIRE's data comprised fairly large numbers, and a serious departure from normal Mendelian expectation was apparent in his second generation, which indicated linkage between the two genes involved. His data, however, were not suitable for a study of this point, and it went unnoticed. In the summer of 1916 the writer, under the direction of Professor CASTLE, undertook the investigation of the relationship between pink-eye and albinism in mice. At that time we were not aware of the work of HALDANE *et al.*, but were familiar with DARBISHIRE's data. This investigation was conducted by the writer until April 1917, and was then taken over by Professor CASTLE, who carried it on until my demobilization in March 1919. A report by CASTLE gave the crossover value between pink-eye and albinism as approximately 14 percent.

The linkage between albinism, pink-eye and red-eye in rats was first noted by CASTLE (1916). It was later established by increased data (CASTLE 1919) from which was calculated a crossover value of 21.1 percent between pink-eye and albinism. This rested on a total of 90 gametes. Only one cross-over was detected between red-eye and albinism in 434 tested gametes. The crossover value between these genes was hence but a fraction of one percent and a very close linkage between them was indicated.

In confirmation of this linkage WHITING and KING (1915) have reported crosses between red-eyed and ruby-eyed-dilute rats. Such crosses produced full-colored gray rats in F_1 (87) and 59 grays, 19 ruby-eyed dilutes and 23 red-eyed yellows in F_2. No double recessives, viz., ruby-reds, appeared in F_2, indicating that no crossing over occurred between ruby and red, and hence that these two genes are closely linked. Ruby-eyed dilution is an allelomorph of albinism and is therefore determined at the same locus as albinism; it should exhibit the same linkage with red-eye as with albinism. The results obtained indicated that this was the case.

The experiments to be reported here are concerned with the linkage relations of pink-eye and albinism in mice and of red-eye and albinism in rats. It is due to the kindness of Professor CASTLE that I am able to include mouse-breeding data gathered by him during my absence from the laboratory.

THE PRESENT INVESTIGATION

Linkage of pink-eye and albinism in mice

a. The characters involved

The albino and pink-eye characters studied by HALDANE *et al.* and by CASTLE in mice, and by CASTLE and WRIGHT in rats, are identical with the characters under consideration in the present report. The Mendelian nature of each has been firmly established by numerous investigators. Albinism has long been known as a unit variation recessive to color in the mouse, rat, guinea-pig and rabbit, to mention only members of the order Rodentia. It consists in complete (or almost complete) absence of color from the hair, skin and eyes, leaving the hair and skin white and the eyes pink. CUÉNOT first attributed this absence of color in the albino to the absence of a chromogen or color base in the presence of which pigment-forming enzymes may produce their respective colors. The gene for color or chromogen has been represented by "*C*," its absence or albinism by "*c*." The genes for pigment-forming enzymes were shown to be present in albinos but to have no effect in the absence of *C*. This has remained the most convenient theory for genetic experiments and the notation arising from it is still used, although its correctness has been seriously questioned. ONSLOW (1915) has found evidence that absence of color in mammals is due not to absence of chromogen but to enzyme differences, in certain specific cases to the absence of a peroxidase. When tyrosinase was added to preparations from albino animals, the oxidation of tyrosin proceeded and black pigment was formed. This agrees well with recent discoveries of graded series of albinos in guinea-pigs, rabbits, rats, cats, and other animals. All of these grades of albinism possess a common gene which may be present in a series of alternative forms, each condition allelomorphic with its fellow members of the series. This central gene for albinism is present not only in animals entirely devoid of color, but also in animals capable of developing a certain amount of black or brown pigment, such as the Himalayan rabbit or the Siamese cat, in which the extremities are pigmented. The explanation of albinism is, then, nothing so simple as mere presence or absence of a gene for color. It is rather, as WRIGHT (1916) has suggested, a graded condition due to the varying activity or inhibition of a basic enzyme on a chromogen. Each grade has shown itself to be due to a gene of an allelomorphic series, the more intense grades being dominant to those of less pigmentation. Thus in guinea-pigs WRIGHT (1916) has identified four multiple allelomorphs of the color gene, (1) intense or full color, (2) dark-eyed dilution, (3) red-eyed dilution, and (4) albinism. The form of albinism found in guinea-pigs should be distinguished from the true pure white albinism of rabbits, rats and mice, for the albino guinea-pig has pigmented extremities like the Himalayan albino rabbit. No ordinary albinism has yet been reported in guinea-pigs. In rabbits, CASTLE, PUNNETT and others have shown that a series of multiple allelomorphs exists from (1) intensity through (2) Himalayan albinism to (3) ordinary albinism. In rats, WHITING and KING (1918) have recently found a ruby-eyed dilute variation due to a gene allelomorphic with intensity and albinism. The allelomorphs of the color gene in rats are now, in order of dominance (1) intensity (2) ruby-eyed dilution (3) albinism.

The experimental breeding of mice has disclosed no such alternative conditions of the color gene as are exhibited in guinea-pigs, rabbits and rats. Unit variations in density of color occur, but in each case these have been traced to genes distinct from that for color.

A second unit variation affecting color has been found in rats, guinea-pigs, and mice. The appearance of animals exhibiting this variation is similar in all three species. The eyes are pink, resembling the eyes of albinos, although a slightly darker shade is discernible on close examination, due to microscopic amounts of pigment in retina and iris. The fur and skin of pink-eyed animals are distinctly lighter in color in those portions which in dark-eyed individuals are black or brown, while yellow portions appear similar in both dark-eyed and pink-eyed animals.

The gene for this character appears to be homologous in all three species in which it is found, for it produces homologous results and has shown in the rat and mouse similar relationships with other genes. It was shown by CASTLE and LITTLE (1909) to be distinct from the intensity-dilution pair of allelomorphs (*D* and *d*) and to be the recessive allelomorph of dark-eye (*P*). By them it was given the symbol (*p*). The work of DARBISHIRE had shown it to be distinct also from the gene for color.

The mode of action of the gene for pink-eye is to reduce materially the number of black and brown pigment granules formed in hair, skin and eyes. Its action is selective, for yellow, which occurs as a non-granular ground color, is not affected in amount or intensity. Pink-eyed black rats and mice are a pale cream color, while in pink-eyed black guinea-pigs the black is still less in amount and the animals are sooty white. Pink-eyed brown mice and guinea-pigs are paler in color than pink-eyed blacks and show a tawny or ochraceous tinge. The pink-eyed black agouti and cinnamon varieties of all three animals are taken by the casual observer to be yellow, since the intense yellow tips of the agouti hairs are as fully colored as in dark-eyed agoutis and overlie the usually black or brown portions of the hairs nearer to the body. In pink-eyed agouti mice, these black or brown areas are pale blue-gray and pale brown respectively, while in rats and guinea-pigs the black or brown pigments have almost entirely disappeared from the hair leaving the bases a dirty cream color and the tips clear yellow. In rats such animals are known as pink-eyed yellows, though when crossed with dark-eyed blacks they soon give evidence of their agouti nature by producing dark-eyed agouti young. A similar quantitative reduction of granular pigments is seen in the eyes of pink-eyed colored animals. The ordinary dark eye of rodents is colored by black and brown pigment granules in retina and iris. In pink eyes Miss DURHAM (1908) found that pigment was not entirely lacking as in albinic eyes, but that iris and retina contained very small numbers of black or brown granules. LITTLE found very little pigment in the retina and a moderate amount in the iris. The pinkness of the eye is due to the reflection of light from unobscured blood vessels of the retina through an almost transparent iris.

b. The plan of the experiments

The plan of the experiments designed to determine the linkage relations between pink-eye and albinism in mice does not differ from the general plan already established for testing linkage relations in insects and plants. Albino females of known genotype (*ccPP*) were crossed with pink-eyed colored males of genotype *CCpp*; and similar matings were made between albino males and pink-eyed colored females. The first generation from these crosses consisted entirely of dark-eyed colored animals of which 142 were raised. These F_1 animals were then mated to each other and a second generation of 580 individuals was recorded. The actual distribution of these F_2 animals is given below contrasted with the distribution expected on an hypothesis of independence of the pink-eyed and albino genes.

	Dark-eyed colored	Pink-eyed colored	Albino	Total	
Actual	295	129	156	580	$\chi^2 = 7.597$[1]
Expected	*326.25*	*108.75*	*145*	*580*	$P = .0231$

The distribution obtained fits the theoretical distribution very badly and shows a distinct excess of pink-eyed and a paucity of dark-eyed. This agrees with the F_2 distribution of DARBISHIRE and of HALDANE, and indicates that repulsion has taken place between (p) and (c) in the gametes of F_1, for by random, independent assortment of these characters among the F_1 eggs and sperm we should obtain the following array of genotypes:

F_1 parents	*CcPp*	×	*CcPp*	
Gametes	*CP*		*CP*	crossover
	cp		*cp*	
	Cp		*Cp*	non-cross-over
	cP		*cP*	
F_2 zygotes expected	1 *CCPP*	1 *CCpp*	1 *ccPP*	
	2 *CCPp*	2 *Ccpp*	2 *ccPp*	
	2 *CcPP*		1 *ccpp*	
	4 *CcPp*			
	9 dark	3 pink	4 albino	

Since the F_2 distribution is manifestly altered by linkage, and since the characters supposed to be linked entered the original cross separately

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[1] In judging the goodness of fit of the distributions obtained to the theoretical distributions required by independent Mendelian assortment I have used PEARSON'S formula $\chi^2 = \Sigma \frac{(o—c)^2}{c}$ and the values of P corresponding to values of χ^2 as calculated by ELDERTON in PEARSON'S "Tables for statisticians and biometricians" (1914). Believing, however, that this method of measuring goodness of fit should not supersede but rather aid the usual method of geneticists in judging by inspection combined with a consideration of the particular biological facts and conditions of the experiment, I have added to each table without comment the values of χ^2 and P which I calculated for my own convenience.

($Cp \times cP$) these combinations should be relatively more frequent; while the combinations CP and cp could occur only as a result of crossing over and should be relatively less frequent.

To measure the frequency of crossing over in the F_1 gametes, the F_2 pinks and albinos were saved and tested. The F_2 pinks were crossed with pure albinos ($ccPP$); the F_2 albinos were crossed with pure pinks ($CCpp$). In this way the F_2 pinks and albinos were sorted into their several biotypes. Out of 75 F_2 albinos thoroughly tested by crossing with pure pink, 55 proved to be of genotype $ccPP$ and 20 of genotype $ccPp$. Of the 150 F_1 gametes which produced these 75 albinos, 110 (2×55) must have been cP (non-crossovers); of the remaining 40 gametes, 20 must have been CP and 20 cp (crossovers). The F_1 crossover gametes were then 20 out of a total of 150 or 13.33 percent.

Similarly, out of 63 F_2 pinks, 45 proved to be of genotype $CCpp$, due to combinations of 90 non-crossover gametes, while 18 proved to be $Ccpp$, referable to 18 Cp (non-crossover) and 18 cp (crossover) gametes. The crossover gametes totalled 18 out of 126 or 14.28 percent. A combination of the figures for both of these tests shows that out of 276 F_1 gametes tested, 38 or 13.76 percent ($\pm$ 1.396) were due to crossing over.[2] We may regard this as a preliminary estimate of the strength of the linkage between these characters.

From pink-eyed individuals shown by the test matings to be $Ccpp$, albino young were obtained which were recessive in both characters, *viz.* albinos pure for pink-eye ($ccpp$). All animals of type $CcPp$, where c and p had entered separately, were also saved, and reciprocal matings of $CcPp$ and $ccpp$ were made in large numbers. Results of these matings gave a direct index of the amount of crossing over in both sexes as may be seen from the possible combinations.

Theoretically, the crossover classes $CcPp$ and $ccpp$ should be equal, and therefore all dark animals resulting from this mating should be equal to one half of the total number of crossovers. Fortunately dark-eyed young may be distinguished at birth from light-eyed (pink-eyed and albino) young by the presence of a distinct ring of black pigment in the position of the eye. This region in pink-eyed and albino young is quite colorless. This fact made it possible to record the numbers of dark-eyed and light-eyed young soon after birth. The young were then killed and the mother bred again. Large numbers were raised by this method in a comparatively short time and in few pens.

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[2] The probable errors of linkage values in this paper have been calculated by use of the formula $E_p = .6745\sqrt{\frac{P(1-P)}{n}}$ as given by HALDANE (1919). P is the observed value of the theoretical linkage value p, and is calculated from the distribution of gametes formed by the double heterozygote in the proportion $\frac{p}{2}AB : \frac{1-p}{2}Ab : \frac{1-p}{2}aB : \frac{p}{2}ab$. Where A and B enter separately, the linkage value is p; where A and B enter together the linkage value is $1-p$.

These matings produced 3141 young, of which 2919 were pink or albino and 222 were dark. Since the darks represented approximately one-half the crossovers, the total crossovers were presumably 444 or 14.14 percent (± .418) of the total.

Calculations of linkage strength had up to this time been based on the relations between the genes *c* and *p* when they entered the cross separately (repulsion). To complete the experiment crosses were made in which the genes entered the cross together (coupling). Dark-eyed animals (*CCPP*) were mated with albinos recessive in both genes, (*ccpp*).

The first generation consisted entirely of dark-eyed animals (*CcPp*) and these were mated again to the double recessives. These matings should yield the following genotypes:

$$\begin{array}{lllll}
\text{Parents} & & CcPp \times ccpp & & \\
\begin{array}{l}\text{Non-crossover}\\ \quad\text{gametes}\end{array} & \left.\begin{array}{l}CP\\ cp\end{array}\right\} & \times\ cp = & \left\{\begin{array}{ll}CcPp & \text{(dark)}\\ ccpp & \text{(albino)}\end{array}\right\} & \begin{array}{c}\text{Non-crossover}\\ \text{zygotes}\end{array} \\
\text{Crossover gametes} & \left.\begin{array}{l}Cp\\ cP\end{array}\right\} & \times\ cp = & \left\{\begin{array}{ll}Ccpp & \text{(pink)}\\ ccPp & \text{(albino)}\end{array}\right\} & \begin{array}{c}\text{Crossover}\\ \text{zygotes}\end{array}
\end{array}$$

Here the distinguishable class (dark) represents one-half the non-crossovers. To find the number of crossovers, twice the number of darks produced must be subtracted from the total number of offspring.

The actual number of animals produced from this cross was 3331 of which 1414 were dark and 1917 were light. The calculated number of crossovers was 503, which is 15.1 (± .417) percent of the total produced.

The final value of the linkage between the genes for pink and albinism in mice is calculated by combining the figures for all experiments as in table 1.

For several reasons this final value may be regarded as expressing very closely the relations between these two characters in mice. The number of observations is large, and the agreement between the values in the separate crosses is close. The probable error (± .288) of the

TABLE I

Experiment	Total	Crossovers	Percent
Repulsion (*Cp* × *cP*)	3142	444	14.13 (± .418)
Coupling (*CP* × *cp*)	3331	503	15.1 (± .417)
Repulsion (F_2)......	276	38	13.76 (±1.396)
Total	6749	985	14.59 (± .288)

total value is extremely small for linkage determinations. The personal error is small with such easily distinguishable characters, and the method of taking records within twenty-four hours after birth has probably reduced the possible error arising from selective depletion of young after birth. In the latter part of the experiments a study of this last-named source of error was made in order to determine whether it had altered the linkage value by affecting the distribution of light and dark young. A brief discussion of this study will show that it has not seriously altered the results.

c. Depletion of litters

It has been noticed by several experimenters with rodents that the size of the litter is subject to a certain reduction from birth until the young animals are weaned. A quantitative study of such reduction or elimination has been made by DETLEFSEN and ROBERTS (1918) and my results bear out their general conclusion. Although a part of this reduction is due to infant mortality, accident and malnutrition, the more important cause is the destruction of young by older animals in the pen, spoken of in the present paper as depletion. The young are eaten either by the male, by other pregnant females in the pen, or by their own mother because of annoyance or overcrowding by other animals. This is due almost entirely to the usual method of breeding several females, (in these experiments three) to one male, and allowing the young to be born in the breeding-pen. Isolation of pregnant females in separate pens has been found to eliminate almost entirely the loss from depletion. To study its effect in these experiments certain mothers from the coupling series were isolated when pregnant. One-hundred and forty-five litters born of these mice in isolation averaged 6.02 young per litter while 145 control litters born in breeding-pens averaged 4.47 young per litter. Has this depletion altered in any way the distribution of the classes of young expected? The only way in which the distribution could be altered is by discrimination on the part of the mother between light-eyed and dark-eyed young, although it is possible that certain character combinations have greater survival value than others. Depletion unaccompanied by discrimination or difference in survival values should affect both light- and dark-eyed classes alike. Data on this point are found in the comparative distributions of isolated (undepleted) litters and breeding-pen (depleted) litters. Of 874 young born in isolation pens 40.3 percent were dark- and 59.7 percent were light-eyed (pink or albino). Of 649 young born in breeding-pens 42.8 percent were dark- and 57.2 percent were light-eyed. Although the distributions are quite similar it appears that the smaller (dark-eyed) class has been depleted somewhat less than the larger light-eyed class. If we consider the effect of such depletion on the number of crossovers, it is apparent that in a larger number of litters such differential depletion has not been operative.

For the 649 young born in breeding-pens the percentage of crossovers observed is 14.3 percent; for the 874 young born in isolation this value is 19.04 percent. The effect of depletion seems to be to reduce the number and percentage of crossovers. The crossover value is however a function of the number of dark-eyed animals produced, since the darks constitute in this experiment (coupling) one-half the non-crossovers. Any cause which tends to increase the proportion of darks, therefore, tends also to reduce the proportion of crossovers. If the differences caused by depletion in the two series of litters given above are real and not due to random sampling, the differences should persist when larger numbers are considered. Let us examine the operation of depletion on 3142 young born in the reciprocal experiment (repulsion series). All animals in this experiment were born in breeding-pens and all litters were subject to depletion. According to the previous data the proportion of light-eyed young should be lowered and the proportion of dark-eyed young raised. Since the darks here constituted one-half the crossovers any cause tending to increase the proportion of darks should increase also the proportion of crossovers. Yet the percentage of crossovers for these depleted litters (14.13) (repulsion series) is practically the same as the percentage for the depleted control litters (14.3) of the coupling series and the same cause should operate in opposite directions in the two series. Where the numbers are small, differential depletion of light-eyed young appears significant, yet where the numbers are large, such a significance disappears. It seems probable, then, that differential depletion has not seriously affected the gross result, and that its appearance in small samples was due to random sampling.

d. Crossing over in both sexes

Records were kept throughout the experiments of the number of crossovers produced by heterozygous males and females respectively. These records are summarized briefly in the following table.

TABLE 2

Repulsion experiment ($Cp \times cP$)	Non-crossovers Cp — cP	Crossovers CP — cp	Total	Percentage of crossovers
F_1 ♂♂ ($CcPp$) × cp	1542	230	1772	12.98 (±.612)
F_1 ♀♀ ($CcPp$) × cp	1155	214	1369	15.63 (±.661)
Coupling experiment ($CP \times cp$)	CP — cp	Cp — cP		
F_1 ♂♂ ($CcPp$) × cp	1638	273	1911	14.28 (±.539)
F_1 ♀♀ ($CcPp$) × cp	1190	230	1420	16.19 (±.652)

Here is conclusive evidence that crossing over takes place in both sexes. Mice, therefore, are to be added to the list of animals in which this phenomenon occurs, which now includes the rat (*Mus norvegicus*), the grouse locust (Apotettix) as well as certain plants, e.g., *Primula sinensis* (ALTENBURG 1916).

The evidence for mice as given above seems to indicate that crossing over takes place more frequently in oögenesis than in spermatogenesis, since relatively more crossover gametes are produced by the F_1 females than by the F_1 males. This difference is of doubtful significance, however, for it is only slightly over three times its probable error. This may be seen from a retabulation of the data in table 2.

TABLE 3

	Non-crossovers	Crossovers	Total	Crossover percentage (p)	E_p	Difference	$\frac{\text{Difference}}{E_{\text{Difference}}}$
All F_1 ♂♂	3180	503	3683	13.65	±.381	2.26±.593	3.81
All F_1 ♀♀	2345	444	2789	15.91	±.466		

Applying three times the probable error as a test of a significant difference is a rigid criterion and does not exclude the possibility of some significance. It does, however, make the significance appear doubtful.

e. Variations in crossover percentages

In all work on linked genes considerable variation in linkage values has been noted between individuals and families as well as between animals of different age and sex and those in different environments. In these experiments the environmental conditions and the feeding were to a considerable degree constant and uniform. It is doubtful whether in warm-blooded animals temperature could have an important effect on the behavior of the chromosomes in gametogenesis, beyond an inhibitive effect of very high or very low temperatures on ordinary breeding activities. Concerning the effect of age only very general statements can be made for no record was kept of exact age of parents at the time that litters were born, and the addition of new young parents to the breeding-pens to replace casualties prevented a close study of this effect. However, in general, there was a very slight and probably insignificant increase in the amount of crossing over from the beginning of the experiments to their completion. Animals were discarded after the climax of their breeding capacity had been reached so it is not known whether old age would have brought about any change in amount of crossing over.

The variation of individual mice and of different pens in amount of crossing over was considerable but this is inevitable where one individual can furnish in its lifetime so small a sample of the whole result. The amount of crossing over was found to vary for individual fathers from 0 to 50 percent for progenies of over 20 young each. Since crossing over is approximately equally frequent in both sexes this variation is probably similar for individual mothers. The same males which had given low or high crossover percentages with certain females did not maintain this percentage with other females. In this as in other linkage determinations these individual differences are probably due rather to the error of sampling than to genetic differences in amount of crossing over, and the results *in toto* are none the less reliable because of the presence of small individual variations.

Linkage of red-eye and albinism in rats

The discovery of linkage between the genes for red-eyed yellow and albinism in rats by CASTLE and WRIGHT (1915) and CASTLE (1916) has provided the material for the experiments here reported, which are in continuation of the work of the above authors. The rapid spread of a serious infestation of parasites caused the practical discontinuance of the previous experiments with rats and has seriously hampered the present investigation.[8]

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[8] The parasite responsible for most of the trouble has now been identified as a minute sarcoptid mite, *Sarcoptis notoedres* (Bourg. and Delaf) var. *muris* (Megnin) which infests the extremities of the rat, chiefly ear, nose and tail. The presence of

In CASTLE's last report (1919) a very close linkage between the genes for red-eye and albinism was indicated and more data on this linkage was sought. The original cross of red-eyed yellow (*CCrr*) × albino (*ccRR*) has been repeated and a large first generation has been raised consisting entirely of dark-eyed, dark-coated animals (*CcRr*). These have been inbred and have produced a second generation of 1494 young distributed as follows:

TABLE 4

	Dark-eyed	Red-eyed	Albino	Total	χ^2	P
Observed ..	737	395	362	1494		
Expected (*c* and *r* independent)	840.38	280.12	373.5	1494	61.09	.0000
Expected (*c* and *r* completely linked) ...	747	373.5	373.5	1494	1.72	.433

The actual distribution is much closer to that expected on an hypothesis of complete linkage than it is to the proportions expected if *c* and *r* are independent. The hypothesis of independence is in fact excluded by the data. If a very small amount of crossing over has taken place, this

this parasite is first noted from the appearance of the pinnae of the ears, near the margin of which may be seen minute swellings in the form of tubercles. These are the nests of eggs which have been laid by the female mite. They soon hatch and the young larvae in enormous numbers begin feeding on the substance of the pinna. They literally eat their way in toward the skull, sucking out the liquid constituents and leaving behind them a mass of dead dry tissue. At this time the ear is characteristically scabby and withered in appearance. The infestation soon spreads to the tail and nose and causes almost entire cessation of breeding activities in the infested rats. The disease is not immediately fatal although it shortens the life of the infested rats and since breeding ceases infested stocks soon die out. Various dips were tested in an attempt to control the spread of the parasite. The most helpful of these was a solution of sodium fluoride (1½ ounces); powdered sulphur (4 ounces); yellow soap (2/3 ounce); warm water (2 gallons). Young rats dipped in this solution at the time of weaning are seldom infested; older rats may sometimes be cured by repeated dippings at intervals of ten days or two weeks, although the older animals are often weakened by the parasite and succumb after dipping. The only satisfactory method was found to be individual applications to the infested parts of warm vaseline containing two percent carbolic acid and enough powdered sulphur to make a workable paste. Although the spread of parasites was never absolutely controlled, the stock was kept in fairly good condition by dipping all rats when weaned and generally once thereafter, killing all cases of bad infestation, sterilizing all pens and using the salve treatment on animals which it was necessary to save.

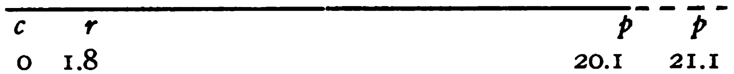
should result in a slight deficiency in the dark-eyed class and a corresponding excess in the red-eyed class, as explained by CASTLE (1919). Both of these conditions are realized in the data, confirming the previous conclusions from tests of F_2 animals.

The red-eyed F_2 class should normally consist of rats of genotypes *CCrr* and *Ccrr* but if *c* and *r* are linked the genotype *Ccrr* could only be formed by crossing over in the gametes of F_1. Similarly the F_2 albinos should normally consist of genotypes *ccRR, ccRr* and *ccrr,* while if *c* and *r* are linked the last two classes could occur only as a result of crossing over. To measure the frequency of crossing over, F_2 reds were therefore tested by mating them with albinos; and F_2 albinos were tested by mating them with red-eyed yellows. Any F_2 red-eyed animal which, when tested in this way, produces albinos, and any F_2 albino which produces red-eyed young, must have developed from an F_1 crossover gamete. Out of 135 F_2 animals thus tested, 130 produced only dark-eyed young (4 or more) while 5 produced dark-eyed and light-eyed (red-eyed or albino) young. The last named young were due to crossing over in 5 F_1 gametes. The total number of F_1 gametes responsible for the production of the 135 tested animals is 2 × 135 or 270. The crossover gametes (5) are 1.8 (± .54) percent of the total gametes and this may be regarded as a preliminary estimate of the linkage strength between the genes for red-eye and albinism in rats.

Arrangement of three linked genes in rats

With quantitative data on crosses involving three linked genes in rats now available, we are in a position to represent graphically the relationships of the genes *c* (albinism), *r* (red-eyed yellow), and *p* (pink-eyed yellow). *Cr* (ruby-eyed dilution) has been shown to be an allelomorph of albinism and is evidently to be placed at the same locus with *c*. Assuming that the "distance" between genes is proportional to the amount of crossing over between them, the albino locus evidently occupies the end or zero position in this system of three. Its crossover value with red-eye is 1.8 ± .54 percent with pink-eye 21.1 ± 2.92 percent, while the red-pink crossover value is 18.3 ± .38 percent. Considering the probable errors of the values involved, the mathematical relations of these three characters are such that the "distance" albinism-red (1.8) plus red-pink (18.3) equals approximately the distance albinism-pink (21.1). To satisfy this relation we may assume that the locus "*r*" is between the loci "*c*" and "*p*" and on the same straight line with them.

Graphically the arrangement of the three linked genes in the chromosome may be plotted to scale as follows:



The most reliable value, that for *r—p* was laid off first; then the next most reliable value (*r—c*) was laid off to the left of *r*. Using *c* as a base the value *c—p* (the least reliable) was measured along *r—p*. This gave the position *p′* which was found to be to the right of *p*, an actual error of 1 unit. But a point can have but one locus. Therefore either *cp′* is too long, or *cr* is too short, or the theory of linear arrangement does not fit the case. The first of these possibilities is the most probable cause of the error since the distance *r—p* is subject to but a small probable error and since the linear arrangement is within the probable errors involved.

The above is essentially the arrangement assumed by MORGAN, STURTEVANT, *et al.* (1915) for the linked genes of Drosophila and the present evidence from mammals appears to provide confirmation of the conclusions already drawn from insect data.

More work is necessary before the rat data can be regarded as forming a *critical* test of the linear arrangement hypothesis. In the discussion above I have used only the new data on the amount of crossing over between *c* and *r*. If to these data there are added CASTLE'S (1919) earlier figures for this linkage (one crossover out of 434) the *c—r* value becomes .85 ± .23 which on the linear hypothesis increases the actual error of *c—p* from 1 to nearly 2. If, then, the three genes *c, r* and *p* in rats are linearly arranged, the crossover value *c—p*, when larger numbers are obtained, will be found to be less than the present figures.

The occurrence of crossing over now makes possible the production of a race recessive in both red-eye and albinism, and when such rats are crossed with animals heterozygous in these genes (*CcRr*) a method of measuring the amount of crossing over directly and on a large scale will be available. Such a doubly recessive race is now in process of production.

Comparative frequency of crossing over in males and females

No direct data on comparative frequency of crossing over in the two sexes are provided by these results. Dr. CASTLE has, however, kindly given me access to his data on the linkage of pink-eye and red-eye in rats. This evidence is presented in condensed form in table 5.

TABLE 5

Comparative frequency of crossing over in male and female rats.

Non-cross-overs	Cross-overs	Total	Crossover percentage (p)	E_p	Difference	$\frac{\text{Difference}}{E_{\text{Difference}}}$
All F_1 ♂ ♂ 1742	321	2063	15.56	±.538	4.90±.75	6.51
All F_1 ♀ ♀ 2134	549	2683	20.46	±.525		

Applying the probable error of the difference in linkage values as a criterion, the greater frequency of crossing over in the female is significant. In mice where crossing over was also found in both sexes, the frequency was somewhat greater in the female than in the male. In mice, however, the difference was of doubtful significance, since it was only slightly greater than three times its probable error. In rats the difference is greater, and is equivalent to about 6.5 times its probable error. The difference is in the same direction in both rats and mice, for in both animals it is the female which produces the larger proportion of crossover gametes. From this it may follow that oögenesis differs from spermatogenesis in the frequency with which the hypothetical exchange of genes takes place.

The occurrence of crossing over in both males and females is characteristic of the only two mammals in which linkage has been studied. This fact deserves special mention, and one might be tempted to conclude that sexual differences in crossing over vary on a quantitative scale in different animals. Thus in some animals crossing over never occurs in the male; in others it occurs very rarely in the male; while in others it occurs regularly but less frequently than in the female. On the other hand we have examples of its occurrence exclusively in the male, and all of these conditions are met with in fairly closely related orders of insects, Diptera, Orthoptera, and Lepidoptera. The same may prove to be true of mammals. Therefore no wide significance should be attached as yet, to the fact of crossing over in both sexes in mammals.

The important point is the occurrence of differences in crossing over in the two sexes, for the logical *a priori* expectation is similarity. The behavior of the autosomes is apparently similar in both sexes during gametogenesis. This is witnessed by such genetic evidence as the data from the Drosophila gynandromorphs which show that the sexes do not differ in autosomal characters, each sex receiving a similar complement of autosomes by apparently similar methods. No crossing over in one sex appears, then, to be the anomalous condition which needs explanation.

It was formerly thought that no crossing over was a peculiarity of that sex which was heterozygous for the sex-chromosome. In Orthoptera, however, the cytological evidence shows that the male is the heterozygous sex and recently crossing over has been reported in male locusts (NABOURS 1919). Likewise in rats and mice the male is apparently heterozygous for the sex element, and the genetic evidence shows that crossing over is independent of this fact. It is more or less apparent why crossing over of sex-linked genes does not occur in the heterozygous sex, for here the sex-chromosome has probably no exact homologue, though even here it may be found that the Y or zero chromosome may carry characters and take part in synapsis. But the fact remains that no crossing over or less crossing over in the autosome cannot be explained by reference to the sex chromosome alone.

The explanation of such puzzling differences is probably attainable only by cytological methods and evidence. The chromosomes appear to be so intimately concerned with linkage and with crossing over that one would naturally look for sexual differences in the structure or functioning of these bodies to explain such sexual differences in crossing over. Such a comparison provides an interesting and difficult problem for cytologists.

SUMMARY

1. The localization of genes in mammals is discussed and possible reasons are given for the comparative infrequency with which linkage has been found in this class.

2. The crossover value between the genes for pink-eye and albinism in mice is estimated to be approximately 14.5 percent based on 6749 observations.

3. Post-natal depletion of litters appears not to have seriously affected the crossover value.

4. Crossing over is found to occur in both sexes in mice, with approximately equal frequency, although there is some evidence that it takes place slightly oftener in oögenesis than in spermatogenesis.

5. The crossover value between red-eyed yellow and albinism in rats is found to be approximately 1.8 percent based on 270 tested gametes. When the present figures are added to those obtained earlier by CASTLE, this crossover value is found to be about .8 percent on 704 tested gametes.

6. The linkage relations of the genes for albinism, red-eye and pink-

eye in rats indicate that these genes are arranged in a linear order in one chromosome.

7. Evidence from a large series of observations by CASTLE, is analyzed, and it is found that in rats crossing over occurs in both sexes, but somewhat more frequently in the female. The sexual difference, measured by the probable error, is significant. It is suggested that since this difference appears in both rats and mice as well as in other animals, it may be due to sexual differences not yet discovered in either the structure or functioning of the chromatin.

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INDEPENDENT GENES IN MICE*

L. C. DUNN
Storrs Agricultural Experiment Station, Storrs, Connecticut

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INTRODUCTION

One of the most fruitful hypotheses of recent research in genetics has been that connected with the localization of the hereditary determiners or genes. Under the name of the chromosome hypothesis of heredity this idea has undergone a rapid development. The residence of genes in the chromosomes is now well established, the main data in support of this theory having come from the experimental study of the linkage relations between genes for Mendelian unit characters. The study of the topography of the germinal material of as many species as possible is a necessary forerunner of a unified knowledge of heredity, and to this end it is advisable that the relationships between known unit characters be thoroughly investigated. Several cases of linkage between genes for color variations in rats and mice are now known and consequently we have some positive knowledge of the localization of certain genes in these species. We may widen this knowledge by the investigation of the relationships between other genes. Even if no additional linkages are discovered we may be informed by the evidence thus accumulated that certain genes are not linked with any others and are therefore presumably determined each in a separate chromosome. The experimental evidence to be presented here is drawn from investigations of this nature on the inheritance of several genes for color variations in mice.

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*The studies on which this paper is based were made at the Bussey Institution, HARVARD UNIVERSITY.

The linkage of the gene for pink-eye with the gene for albinism in mice (CASTLE 1919, DUNN 1920 a) established the identity of one chromosome. Using this as a base it was considered important to find out whether any other color characters were determined in this chromosome. Either pink-eye or albinism could be used as a reference character, and crosses of either of these with other characters should discover whether certain other genes were located in this chromosome or should be referred to other chromosomes. For this purpose it was decided to use pink-eye (*p*) rather than albinism because its presence as well as the presence of other somatic characters with which its relation was to be tested is immediately discernible, while albinism does not allow the genotypic content to express itself because albinos develop neither color nor pattern.

THE RELATION OF PINK-EYE AND PIEBALD

The first character tested for location in the albino chromosome was piebald or recessive spotting, for which the symbol "*s*" is used.[1] Its normal dominant allelomorph is self (symbol *S*). Piebald was selected because there was no significant amount of data on the relations of pink-eye and piebald. Pink-eye had shown no linkage with agouti, yellow, black or brown in the data of LITTLE (1913), LITTLE and PHILLIPS (1913), and DETLEFSEN and ROBERTS (1917), and the numbers involved were large, so that it was not thought necessary to repeat the experiments.

A description of the character piebald in mice may be found in the works of CUÉNOT (1902 to 1911) and LITTLE (1915), and an account of its variability by the author (DUNN 1920 b). A brief description will therefore suffice for our present purposes. Piebald mice are spotted with white on a ground of any color. The white areas vary considerably in extent from a small ventral patch covering 12 percent or more of the belly to a condition in which the dorsal surface is 50 percent white and the belly 85 percent white. The distribution of the white areas follows no such definite pattern as the Dutch markings of belted cattle or the Dutch spotting of rabbits, with which characters the piebald of mice is in other respects quite similar. An approach to a belt of white about the center of the body and a "white face" or "blaze" condition is often seen, due perhaps to modifiers of the central gene for piebald. The gene for piebald determines only the spotted condition as contrasted with self, while the amount and distribution of the spotting appears to be determined by other modifying genes not yet analysed (LITTLE 1917, DUNN 1920 b). When piebald is crossed with self only self mice result, with an occasional very small spot of white. Such mice interbred, produce on the average three self mice to one piebald, showing that piebald differs from self in one cleanly segregating character.

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[1] The established symbol for piebald spotting has been "*sp*" (LITTLE 1915). It has been considered better to denote this character by only one letter, following the more general custom of Mendelian nomenclature; especially since the occurrence of "*sp*" in formulae containing "*p*" (pink-eye) is likely to be confusing to the reader.

The stocks available at the beginning of the experiment were dark-eyed piebalds (*PPss*) and pink-eyed self mice (*ppSS*). The genes *p* and *s* therefore entered the experiment separately, in different parents. Matings were made between dark-eyed spotted males and pink-eyed self females and an equal number of matings was made between dark-eyed spotted females and pink-eyed self males. The first generation from these reciprocal crosses was the same in both cases. It consisted of 557 self mice, all dark-eyed with the exception of 52 which came from dark-eyed spotted parents heterozygous in the gene for pink-eye (*Ppss*). The dark-eyed self F_1's were presumably of genotype *PpSs* and these heterozygotes were mated *inter se* to produce the second generation. The second generation consisted of a total of 774 animals whose distribution is given below compared with the distribution expected if the genes *p* and *s* assort independently in the heterozygote.

TABLE 1

F_2 generation from cross of pink-eyed self (pS) × dark-eyed piebald (Ps).

	Dark-eyed self (*PS*)	Pink-eyed self (*pS*)	Dark-eyed piebald (*Ps*)	Pink-eyed piebald (*ps*)	Total
Observed	436	145	150	43	774
Expected	*435.4*	*145.1*	*145.1*	*48.4*	*774*

$\chi^2 = .14496$ P = approximately 1[2]

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[2] The mathematical criterion used for judging goodness of fit of an observed to a hypothetical distribution is that of PEARSON ($\chi^2 = \Sigma \frac{(o-c)^2}{c}$) aided by ELDERTON'S values of P corresponding to values of χ^2. The "expected" or hypothetical distribution is in each case that required by independent (non-linked) assortment of genes.

The actual distribution is extremely close to the expected and apparently the F_1 heterozygotes formed gametes *PS, pS, Ps* and *ps* in numbers so nearly equal that the chance that *p* and *s* are linked is practically zero. Nevertheless a further test for even an extremely small amount of linkage was made by mating the F_1 heterozygotes (*PpSs*) to the double recessive pink-eyed piebalds (*ppss*) obtained in the second generation.

If *p* and *s* are independent the heterozygote *PpSs* should form equal numbers of four sorts of gametes, *PS, ps, Ps* and *pS* which if united with *ps* gametes from the double recessive, should produce equal numbers of the four possible combinations of pink and piebald. This cross actually produced 1172 young falling into the following distribution as compared with the distribution expected.

TABLE 2

Back-cross of F_1 [from cross of pink-eyed self (pS) × dark-eyed piebald (Ps)] to pink-eyed piebald (ps).

	Dark-eyed self (*PS*)	Pink-eyed self (*pS*)	Dark-eyed piebald (*Ps*)	Pink-eyed piebald (*ps*)	Total	
Observed	291	315	308	258	1172	χ^2 = 6.61
Expected	*293*	*293*	*293*	*293*	*1172*	P = .087

The distribution observed deviates widely from that expected if *p* and *s* are independent. Its salient features are an excess of self mice (606) over piebald (566); of dark-eyed (599) over pink-eyed (573) and the consequent deficiency of the pink-eyed piebald class. This class was deficient also in the F_2 distribution and it is probable that these departures from expectation are due to one cause, viz., the grading of certain pink-eyed piebalds as selfs, especially since the pink-eyed self class exhibits the greatest excess. The contrast between white and colored spaces is sometimes very slight on young pink-eyed mice, especially when dilution is present. Some piebald mice are spotted only on the belly and as this region is usually lighter in color, the contrast is even less and a few errors would be likely to occur.

The new combinations (crossovers) number 549 while the grandparental combinations (non-crossovers) number 623.[8] One of the non-crossover classes, pink-eyed self, has probably been factitiously enlarged at the expense of the crossover class pink-eyed piebald, while the other non-crossover class is more nearly normal. These facts considered in connection with the normal distribution of the second generation, which according to HALDANE is, in the case of repulsion, nearly as sensitive a test of linkage as the back-cross, make it seem probable that no linkage occurs between the genes for pink-eye and for piebald.

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[8] If this inequality be explained by linkage the crossover value may be computed as 46.84 percent which is subject to a probable error of ± .97 (cf. HALDANE 1919, and DUNN 1920 a). The difference between this crossover value and the normal value of 50 percent, expected if no linkage occurs, is 3.16 which is 3.2 times the probable error. Since the departure from expectation appears to be largely due to errors in grading it is not regarded as sufficient to exclude the hypothesis of independence of *p* and *s*.

RELATION OF PINK-EYE AND NON-AGOUTI

Another relationship of the gene for pink-eye was tested incidentally in the crosses reported above, and the data may be added to the evidence already obtained on this point by LITTLE and PHILLIPS (1913), and DETLEFSEN and ROBERTS (1917). Certain F_1 mice (*AaPp*) were mated with mice recessive in both of these genes (*aapp*), viz., pink-eyed blacks or browns. From such crosses four types of young are expected, and if *a* and *p* are independent these types should be equally numerous. The observed and calculated distributions follow.

TABLE 3

Back-cross of F_1 [from cross of pink-eyed agouti (Ap) × dark-eyed non-agouti (aP)] to pink-eyed non-agouti (ap).

	Dark-eyed agouti	Pink-eyed agouti	Dark-eyed non-agouti	Pink-eyed non-agouti	Total	
Observed	248	234	240	213	935	$\chi^2 = 2.87$
Expected	*233.75*	*233.75*	*233.75*	*233.75*	*935*	$P = .40$

The distribution obtained conforms fairly closely to that expected if *a* and *p* are independent genes. The number of new combinations (crossovers, *AP* and *ap*) is 461 which is very nearly equal to the number of grandparental combinations (non-crossovers, *Ap* and *aP*) which is 474. Evidently the distribution as regards linkage only fits the hypothesis of independence. There is however a marked deficiency in the double recessive class, pink-eyed non-agouti. This appears to be due to the fact that the total agoutis (482) outnumbered the total non-agoutis (453) while the total dark-eyed mice obtained (488) outnumbered the total pink-eyed mice (447). This same phenomenon was in evidence in the larger series of young from this cross recorded by DETLEFSEN and ROBERTS. The same cause to which these authors ascribed the distorted ratio appears to have been operative in the present experiment, namely, differential mortality in the litters between birth and the time of recording young (two weeks) resulting in a greater survival of agouti and of dark-eyed young.

RELATION OF NON-AGOUTI AND PIEBALD

Other incidental data have been extracted from the data dealing with the inheritance of pink-eye and piebald. The original cross was between agouti self (*AS*) mice and non-agouti piebalds (*as*). The genes *a* and *s* should then show coupling in the F_1 heterozygotes if there is linkage between *a* and *s*. Heterozygotes from this cross were agouti and self (*AaSs*). These were mated with non-agouti piebalds (*aass*). From this cross issued the following distribution:

TABLE 4

Back-cross of F_1 [*from cross of agouti self* (*AS*) × *non-agouti piebald* (*as*)] *to non-agouti piebald* (*as*).

	Agouti self (*AS*)	Agouti piebald (*As*)	Non-agouti self (*aS*)	Non-agouti piebald (*as*)	Total	
Observed	221	179	183	200	783	$\chi^2 = 5.60$
Expected	*195.75*	*195.75*	*195.75*	*195.75*	*783*	P = .13

The fit of the observed distribution to that expected if *a* and *s* are not linked is not good. It is not, however, so poor as to exclude the interpretation of the results on the basis of independent random assortment of the two genes concerned. The frequencies of the observed classes deviate from the normal in a definite direction. The original combinations (non-crossovers, agouti self and non-agouti piebald) are in excess of the new combinations (crossovers, agouti piebald and non-agouti self); actually these classes are as 421 : 362. This distortion does not appear to have been due to any serious inequalities between the agouti and non-agouti classes for these are nearly equal 400 : 383 (expected 391.5 : 391.5). Nor is the aberrancy due to serious excess of selfs over piebalds. Actually these classes are as 404 : 379, and the discrepancy is equally distributed between crossover and non-crossover classes. The salient feature is undoubtedly the greater frequency of the original combinations over the new, and if this discrepancy is significant, it indicates linkage between non-agouti and piebald.

To measure the significance we may assume provisionally that *a* and *s* are linked, and calculate the crossover value from the distribution observed. The crossover classes *As* and *aS* total 362 which is 46.23 percent of the total gametes formed by F_1 (783). This value has a probable error of ± 1.20. If *a* and *s* are not linked the crossover and non-crossover classes should be equal, that is, the crossover value should be 50 percent. The deviation of 46.23 percent from 50 percent is 3.1 times the probable error. Applying YULE's criterion that three times the probable error indicates a significant deviation, the departure from expectation in the present case may be significant. Apart from purely mathematical measures of significance, the following facts point to linkage. The classes agouti and non-agouti occur with equal frequency as expected; an error in grading some piebalds as selfs would in this case increase equally one non-crossover class and one crossover class so that such an error if it occurred would be compensatory. On the other hand, there are considerations which should make us chary of stating from these data that linkage occurs between non-agouti and piebald. The data were gathered for another purpose and not with the primary intention of testing the relationships between non-agouti and piebald. The large class in the distribution is agouti self which may have a greater survival value than other classes. Finally from a mathematical standpoint the number of the animals bred is small and the linkage value is close to 50 percent and has a large probable error, while the method of measuring a significant deviation by thrice the probable error is at best approximate. Therefore it is thought best to hold the conclusion in abeyance until more animals are added to the distribution. All crosses bearing on this point are being continued and it is hoped that additional data will be available soon.

NON-AGOUTI—ALBINO CROSSES

A few crosses were made in repetition of a cross reported by HAGEDOORN (1914) who bred agoutis heterozygous for agouti and albinism (*AaCc*) to albinos which lacked the agouti factor (*aacc*). HAGEDOORN recorded only agoutis (*AaCc*) and albinos (*aacc*) from this mating and assumed in explanation that *a* and *c* were completely linked showing in this case complete repulsion. LITTLE (1914) in a criticism of HAGEDOORN's interpretation pointed out that the results obtained appeared, in the light of other experiments, to be anomalous. I have repeated HAGEDOORN's experiment using mice of the same genetic constitution as those used by him. The double heterozygote (*AaCc*) mated to the double recessive (*aacc*) has produced to date 14 agoutis (*AC*), 10 non-agoutis (*aC*), and 22 albinos (*Ac* and *ac*). The expectation if *a* and *c* are independent (11.5 agoutis: 11.5 non-agoutis: 23 albinos) is hence closely approximated, whereas on HAGEDOORN's hypothesis no agoutis should be produced. This evidence is small in numbers but sufficient to show that *a* and *c* are not completely linked and that HAGEDOORN's hypothesis is not applicable.

THE RELATIONSHIPS OF THE GENE FOR BLACK-EYED-WHITE WITH OTHER GENES IN MICE

The data considered in the ensuing paragraphs deal for the most part with the variety of mice known as black-eyed-whites, in which pigment occurs in very limited amounts including usually only eyes, head, and rump while the rest of the pelt is white. Superficially, some of these mice resemble albinos, for the spotting may be so extreme as to produce an entirely white mouse, but somatically and genetically this variation is distinct from albinism, for the eyes are always colored, while the eyes of albinos are pink and entirely devoid of pigment; further LITTLE has found that the gene for black-eyed-white is not allelomorphic with that for albinism but separate and distinct.

After some preliminary work in this variation by CUÉNOT (1903-1911), Miss DURHAM (1908), and MORGAN (1909), LITTLE (1915) succeeded in analysing it. Black-eyed-white spotting he found to be a distinct variation and not merely the extreme of a continuous series in which piebald and self were the darker members. Crossed with piebald, black-eyed-white produces only black-eyed-white and piebald young in equal numbers; crossed with black-eyed-whites like itself, it produces black-eyed-white and piebald in the ratio of 2 : 1; crossed with self mice it produces two types in equal numbers. One of these types is spotted with white in an irregular and varying distribution; the other type is self or nearly so. The first of these LITTLE has called type "A" and the second is his type "B." From such experimental data LITTLE concluded that the black-eyed-white variation was due to a gene independent of and supplementary to the gene for piebald spotting. In crosses this gene behaved as the dominant allelomorph not of piebald or of self, but of something which can only be defined as not-black-eyed-white. To represent the gene for black-eyed-white LITTLE used the symbol "*W*," and for its normal allelomorph "*w*." Black-eyed-whites have been found not to breed true. They always give both black-eyed-whites and piebalds. They are "unfixable" heterozygotes, like yellows and must be represented by *Ww*. They are however, homozygous for piebald which LITTLE represented by the symbol "*sp*" ("*s*" in the present notation"). The formula for black-eyed-white is then in full *Wwss*. When mated together, chance combinations of their gametes should produce some zygotes pure for *W*. LITTLE (1919) later found evidence that such zygotes were formed but died *in utero* and hence were missing from the animals born. This heterozygous nature of black-eyed-whites explains why two types of young are produced in crosses with self. The spotted young (type A) are due to a union of the gamete containing *W* with the self gamete and are genetically *WwSs*. Both gametes produced by the black-eyed-white contain *s*, because type A interbred has produced black-eyed-white, type A, piebald and self, while type B interbred has produced only self and piebald, and has proved to be an ordinary heterozygote between piebald and self (*Ss*).

The relation of black-eyed-white spotting and piebald spotting

Interest in the relationships between these alternative conditions of coat patterns in mice leads naturally to the question, If black-eyed-white, piebald and self are not mutually allelomorphic, how are they related? The evidence is conclusive (*vide supra*) that piebald and self are allelomorphic; that is, that these alternative conditions are determined at the same locus in the same chromosome. Where with reference to this locus are we to place black-eyed-white? If at the same locus, *W* and *s* (or *S*) could never enter the same gamete. But since in the cross black-eyed-white × self, *W* and *s*, and *W* and *S* are known to have entered the same gamete, this cannot be true. *W*, therefore, is determined at a different locus. If this is in the same chromosome with the *S—s* locus, then *W* and *s*, when entering the cross together, should tend to remain together, and when entering separately, should tend to remain separate. In other words, these two genes should give evidence of being linked. If in different chromosomes, *W* and *s* should assort independently and give no evidence of linkage. The critical test of this relationship is the cross of black-eyed-white with self. Here *W* and *s* enter the F_1 zygote to form type A, which we may represent as *WwSs*. To test the gametes formed by this zygote it should be mated to the double recessive, piebald (*wwss*). If *W* and *s* are independent, F_1's of type A should form four types of gametes in equal numbers, (1) one with both *W* and *s*, (2) one with neither *W* nor *s*, (3) one with *W* and lacking *s*, (4) one with *s* and lacking W. If *W* and *s* are linked, types 3 and 4 can only be formed by crossing over and should then be less numerous than 1 and 2. When this back-cross is made four types of young are produced as follows: black-eyed-whites, type A, piebald and self. Black-eyed-whites and selfs are easily differentiated and form the theoretical non-crossover classes. Type A and piebald are subject to considerable overlapping, are hard to distinguish, and taken together, should give the total crossovers. My figures for this distribution are as follows:

TABLE 5

Black-eyed-white (Wwss) × self (wwSS). Test cross of F_1 *type A (WwSs) × piebald (wwss).*

	Black-eyed white (*Wwss*)	Piebald (*wwss*) Type "A" (*WwSs*)	Self (*wwSS*)	Total	
Observed	114	218	117	449	$\chi^2 = .416$
Expected	*112.25*	*224.5*	*112.25*	*449*	P=practically 1

Mathematically, the fit of the actual distribution to that expected if *W* and *s* are independent genes, is extremely good, and excludes any other interpretation for the relationship of these genes. It follows that the loci of the genes for these two kinds of spotting in mice are presumably in different chromosomes.[4] This forms an interesting contrast with the conditions obtaining in rabbits, as recently reported by CASTLE (1919). In rabbits the genes for English (dominant) spotting and for Dutch (recessive) spotting appear to be properties of a single locus. CASTLE's evidence shows them to be either allelomorphic or completely linked. The piebald spotting of mice and the Dutch spotting of rabbits appear to be homologous variations similar in appearance and in genetic cause. The black-eyed-white spotting of mice and the English spotting of rabbits are different in appearance and this difference is reflected in the distinctness of the genes by which they are determined. The fact that both are dominant forms of spotting is thus seen to be an entirely superficial resemblance.

Investigation of types of spotting

Before proceeding to the study of relationships of the gene for black-eyed-white with other genes it was found necessary to study the variability of the various types of spotted mice, in order that the ranges of each type of spotting might be established and that animals resulting from crosses of black-eyed-white with other forms might be classified correctly. This study showed that there was more variability in the expression of the gene for black-eyed-white than had been thought previously. It was found, for instance, that black-eyed-white mice may exhibit nearly as much pigment as piebalds, and that the heterozygotes containing the genes for black-eyed-white, piebald and self (type A) are indistinguishable somatically from piebalds and in some cases from selfs. The results of this study have only an indirect bearing on the localization of genes and are therefore presented in a separate report (DUNN 1920 b). All mice bred in the course of the experiments treated hereafter were carefully graded and classified according to the principles established by the study referred to above.

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[4] These are essentially the conclusions indicated by LITTLE's (1917) data. The present work is not intended merely as supplementary to his but has a distinct object, the investigation of possible linkages in mice.

The relation of black-eyed-white spotting and agouti

LITTLE (1917 b) has presented evidence indicating the independence of the genes for black-eyed-white and yellow. This relationship may be tested either by crosses of black-eyed-white with yellow or by crosses involving black-eyed-white and agouti, since agouti , non-agouti and yellow are allelomorphs and should bear the same relationships to other genes. The latter method was chosen for the present study since LITTLE by crosses directly involving yellow and black-eyed-white had established the important fact that the lethal element connected with back-eyed-white was distinct in origin and effect from the lethal of yellow, and that consequently litters in which both yellow and black-eyed-white were present, were subject to reduction from two sources, due to the prenatal mortality from both of these lethals. The cross of non-agouti black-eyed-whites with agouti self introduces only one lethal and should therefore produce larger litters.

Crosses were made between pure wild agouti mice (*AASSww*) and non-agouti black-eyed-whites (*aassWw*). All F_1 young were agouti (83). Approximately half (42) were self (*AaSsww*), and half (41) were spotted type A (*AaSsWw*). The spotted young were heterozygous for agouti and for black-eyed-white, having received one gene from one parent and the other gene from the other parent. The gametes formed by this heterozygote should then be as regards *a* and *W*, (1) *Aw* (2) *aW* (3) *AW* (4) *aw*. If *A* and *W* are independent, all four sorts of gametes should be equally numerous. If *A* and *W* are linked they should in this case show repulsion, resulting in relatively fewer of sorts (3) and (4), for the latter could only be due to crossing over. To discover which of these contrasted hypotheses is the true one, the F_1 "type A" mice (*AaSsWw*) were crossed with mice recessive in both genes, black or brown piebalds (*aassww*). There resulted six visibly different classes of young, in the following proportions.

TABLE 6

Cross of non-agouti black-eyed-white (aassWw) × agouti self (AASSww); test cross of F_1 type "A" (AaSsWw) × non-agouti piebald (aassww).

	Non-agouti b.-e.-white *aW*	Agouti spotted *AW* and *Aw*	Non-agouti spotted *aW* and *aw*	Agouti self *Aw*	Non-agouti self *aw*	Total
Observed	41	41	76	41	43	306
Expected	*38.25*	*38.25*	*76.5*	*38.25*	*38.25*	*306*

The mice classified as "spotted" are all similar in appearance to piebalds and type A in amount of spotting, and consist as is known from experiments on spotting alone, of two genetically distinct classes *WwSs* and *wwss*. The first of these is type A and the second is piebald, but the somatic expression of these genetic combinations is so similar that they cannot be distinguished directly. Therefore they have been classed together. The distinguishable crossovers are represented by only two classes, agouti black-eyed-white (*AW*) and non-agouti self (*aw*). The distinguishable non-crossovers are non-agouti black-eyed-white (*aW*) and agouti selfs (*Aw*). The sum of the visible crossover classes (84) is found to be approximately equal to the sum of the visible non-crossovers (82). In each of the spotted classes are contained both crossovers (*AW* and *aw*) and non-crossovers (*aW* and *Aw*) and it must be supposed that these occur in the same frequency as in the immediately visible classes. The total of the "spotted" is 140, composed presumably of 70 crossovers and 70 non-crossovers. The calculated total crossovers are then 84 plus 70 or 154 and the calculated total non-crossovers are 82 plus 70 or 152.

A few crosses were made between non-agouti black-eyed-whites and agouti selfs which carried the gene for piebald. From such crosses resulted a few agouti black-eyed-whites which were heterozygous for agouti and black-eyed-white (*AassWw*), *A* and *W* having entered separately. These lacked the gene for self and therefore offered better material for testing for linkage between *A* and *W* since when crossed with non-agouti piebalds only black-eyed-whites and piebalds resulted, and these classes can be immediately distinguished. The frequency of the combinations *Aw, aW* and *aw* in the gametes of the heterozygote can therefore be calculated directly from the somatic ratio in which the young are produced.

Such tests produced the following distribution:

TABLE 7

Cross of non-agouti black-eyed-white (aassWw) × agouti self carrying piebald (AASsWw). Test cross of F_1 agouti black-eyed-white (AassWw) × non-agouti piebald (aassww).

	Agouti b.-e.-white *AW*	Agouti piebald *Aw*	Non-agouti b.-e.-white *aW*	Non-agouti piebald *aw*	Total
Observed	28	27	25	32	112
Expected	*28*	*28*	*28*	*28*	*112*

If linkage occurs between *A* and *W* the two genes should in this case show repulsion. On this basis the crossovers (*AW* and *aw*) number 60 while the non-crossovers (*Aw* and *aW*) number 52.

In all the tests reported above the two genes *A* and *W* entered the experiments separately (repulsion). To complete the test, crosses were made in which the two genes entered the experiment together, and if they are linked they should in this case exhibit coupling. From the test crosses of animals heterozygous in agouti and black-eyed-white there were produced a number of agouti black-eyed-whites (*AassWw*) which had received *A* and *W* from the same parent. These were tested by mating them with non-agouti piebalds. These matings have produced but few young at present but are being continued.

TABLE 8

Test cross of agouti black-eyed-white (AassWw, A and W enter together) × non-agouti piebald.

	Agouti b.-e.-white (*AW*)	Agouti piebald (*Aw*)	Non-agouti b.-e.-white (*aW*)	Non-agouti piebald (*aw*)	Total
Observed	36	22	22	34	114
Expected	*28.5*	*28.5*	*28.5*	*28.5*	*114*

The crossovers in this case are *Aw* and *aW* of which there are 44, while the non-crossovers (*AW* and *aw*) number 70. In the light of the evidence from the preceding tests the excess of the non-crossovers in the last cross is probably due to the dearth of numbers.

Combining the figures from all test crosses involving both *A* and *W* we have the following totals: non-crossovers 274; crossovers 258; total 532. If *A* and *W* are not linked these classes should be equal, 266: 266. The closeness with which the observations approximate this last expectation indicates that probably no linkage occurs between *A* and *W* and that they are hence located in different chromosomes.

The relation of black-eyed-white spotting and pink-eye

Late in the experiments it was decided to test the relationship of the gene for black-eyed-white with that for pink-eye, to determine whether these two genes were linked or independent. DETLEFSEN (1916) had shown that these two genes were distinct and that in the second generation from a cross between them there were produced "pink-eyed-white" mice which carried the color factor. Somatically some of them resembled albinos, but the likeness ended with the pink eyes, since pigment characteristic of pink-eyed mice was present in the same parts which are pigmented in black-eyed-white mice. Genetically these mice were *ppssWw,* while full-colored dark-eyed black-eyed-whites are *PPssWw.* The possible production of such pink-eyed animals resembling albinos or with very small amounts of pale color, required a modification in the plan of the experiments and a test was sought whereby the presence of *W* in a pink-eyed animal might be manifested by some condition other than the black-eyed-white pattern. The cross of a dark-spotted type (*SSWw*) in which *W* produces a small amount of white because acting on self, with piebald (*wwss*) produces two types of offspring. One is type A spotted (*SsWw*) and the other is self (*Ssww*). The inheritance of *W* is hence traceable somatically with a smaller chance of error in classification since only two types are produced. Consequently dark-spotted animals which were also heterozygous for the gene for pink eye (*PpSSWw*) were crossed with pink-eyed piebalds recessive in these three genes (*ppssww*). The genes *P* and *W* had entered the heterozygote together and should hence show coupling if they are linked. This cross should in that case produce relatively more of the combinations *PW* and *pw* (dark-eyed spotted and pink-eyed self) and relatively fewer of the combinations *Pw* and *pW* (dark-eyed self and pink-eyed spotted). If *P* and *W* are independent, equality of these four sorts is expected. The cross has produced young as follows:

TABLE 9

Test cross of mice heterozygous in black-eyed-white and pink-eye (PpSSWw) × pink-eyed piebalds (ppssww).

	Dark-eyed spotted (*PW*)	Dark-eyed self (*Pw*)	Pink-eyed spotted (*pW*)	Pink-eyed self (*pw*)	Total
Observed	16	21	22	23	82
Expected	*20.5*	*20.5*	*20.5*	*20.5*	*82*

Equality of the four sorts of gametes and hence independence of *W* and *P* are indicated. The numbers are however too small to be significant. The total crossovers (*wP* and *Wp*) are 43; the total non-crossovers (*WP* and *wp*) are 39.

Although it is believed that the cross, the results of which have just been presented, is the proper and most accurate method of testing the relation between black-eyed-white and pink-eye, the results of other tests show that there is less danger of confusion between "pink-eyed whites" and albinos than had been feared. As a matter of fact no albinos appeared in the experiments concerned with this linkage, all the young having a discernible amount of pigment in the fur. Therefore the results of other crosses involving *W* and *P* are sufficiently accurate to be included in the totals.

Dark-eyed animals of type A (*PpSsWw*) had resulted from crosses of pink-eyed white (*ppssWw*) with dark-eyed self (*PPSSww*). The genes *W* and *P* had then entered the heterozygote separately and if linked should show repulsion. The young produced by matings of such heterozygotes with the double recessive, pink piebald (*ppssww*) are given in the first line of the following table. Similar tests were made using as the double heterozygote type A's which had received *W* and *P* from the same parent (coupling). The results of this cross are given in the third line of the table.

TABLE 10

Tests of type "A" mice heterozygous in W and P (PpSsWw) × pink-eyed piebalds (ppssww).

	Black-eyed white (*PW*)	Pink-eyed white (*pW*)	Dark-eyed spotted (*PW* and *Pw*)	Pink-eyed spotted (*pW* and (*pw*)	Dark-eyed self (*Pw*)	Pink-eyed self (*pw*)	Total
P and *W* enter together	9	5	20	11	8	9	62
Expected	7.75	7.75	15.5	15.5	7.75	7.75	62
P and *W* enter separately	11	4	18	9	7	10	59
Expected	7.37	7.37	14.76	14.76	7.37	7.37	59

The immediately distinguishable non-crossovers in the first mating are *PW* and *pw*, total 18; the crossovers are *Pw* and *pW*, total 13. The occurrence of these combinations in the "spotted" classes may be assumed to be in the same proportions (18: 13) and the 31 spotted may then be divided roughly into 18 non-crossovers and 13 crossovers. Similarly in the second mating the observed non-crossovers *Wp* and *wP* are 11, and the observed crossovers are 21. The calculated non-crossovers in the "spotted" classes are 9 and the calculated crossovers are 18.

One other series of matings remains which may be added to the above totals. This consisted of test crosses of black-eyed-whites carrying the gene for pink-eye (*PpssWw*, *W* and *P* entering separately) with pink-eyed piebalds (*ppssww*).

TABLE 11

Test cross of black-eyed-white heterozygous in P and W (enter separately) × pink-eyed piebald.

	Black-eyed white (*WP*)	Pink-eyed white (*Wp*)	Dark-eyed piebald (*wP*)	Pink-eyed piebald (*wp*)	Total
Observed	21	21	17	21	80
Expected	*20*	*20*	*20*	*20*	*80*

Here all classes are immediately distinguishable. The non-cross-overs (*Wp* and *wP*) number 38 and the crossovers (*WP* and *wp*) number 42.

Adding together the numbers of non-crossovers and crossovers from all the matings:

TABLE 12

	Non-crossovers	Crossovers	Total
W and *P* enter	39	43	82
together	36	26	62
W and *P* enter	20	39	59
separately	38	42	80
Totals	133	150	283

The totals obtained are too small to afford a final test of the hypotheses of linkage or independence of *W* and *P*. The appreciable excess of crossovers nevertheless creates a strong presumption in favor of regarding these two genes as independent.

SUMMARY

The object of the experiments here reported was to determine through an experimental investigation of linkage relations, the loci of certain genes for color variations in mice.

The following results have been obtained.

1. Pink-eye and piebald spotting segregate from the heterozygote at random in a total of 1946 observations. Their genes are probably not linked but are located in different chromosomes.

2. Additional evidence is added to that already extant, confirming the independence of pink-eye and non-agouti.

3. Evidence comprising 783 observations indicates a slight linkage 46.23 percent ± 1.2 between the genes for piebald spotting and non-agouti. The departure of the observed ratio from that expected if these genes are independent is however of doubtful significance.

4. Black-eyed-white spotting and piebald spotting are neither linked nor allelomorphic, but are properties of distinct loci in different chromosomes. This conclusion, following a preliminary and similar one by LITTLE, is based on 449 direct observations.

5. Black-eyed-white and agouti segregate purely at random and show no evidence of linkage in 532 observations.

6. Black-eyed-white and pink-eye in a short series of data (283 observations) appear to be due to genes independent in heredity and probably located in different chromosomes.

7. Positive evidence is presented which indicates that HAGEDOORN'S hypothesis of complete linkage between non-agouti and albinism is either erroneous or applicable only to his data as a special case.

CONCLUSIONS

No new cases of linked genes in mice have been added to the one already investigated, viz., albinism and pink-eye. Other genes which the evidence indicates are *not* located in the albino chromosome are (1) non-agouti, and therefore its allelomorphs agouti, yellow and light-bellied agouti; (2) piebald spotting; (3) black-eyed-white spotting. The probabilities are that albinism and pink-eye constitute one group; the agouti allelomorphs are the only certain representatives of a second to which possibly piebald may also belong; while a third is represented only by black-eyed-white spotting, and a fourth by piebald spotting (if it proves not to be linked with agouti). Thus four groups of genes are provisionally established, representing four of the 19 autosomes of mice. The work of other investigators (LITTLE, PHILLIPS, DETLEFSEN and ROBERTS) showed that black and brown may constitute a fifth group and that dilution is probably not linked with agouti, black or pink-eye. The position of dilution with reference to other genes, and the position of waltzing are still to be determined.

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THE BEHAVIOR OF BRONCHIAL ASTHMA AS AN INHERITED CHARACTER[1]

JUNE ADKINSON

Peter Bent Brigham Hospital, Boston, Massachusetts

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In a study of 400 cases of bronchial asthma, some attention was given to the behavior of the disease as an inherited character. In the series of 400 cases, 192 or 48 percent gave a history of the occurrence of asthma among other members of the family, and 208 cases, or 52 percent, gave a negative family history. Of the group of 191 patients who were sensitive to proteins as demonstrated by positive skin tests (WALKER, 1916), 96, or 24 percent, of the 400 cases gave a history of the occurrence of asthma or hay-fever in the family, and in 85 cases, or 21.25 percent, the family history was negative. Of the 209 cases which were negative with all the proteins tried in the skin tests, 96, or 24 percent, reported asthma among other members of the family, and 123, or 30.75 percent, knew of no relative who had asthma or hay-fever. In the group of cases sensitive to proteins, 52 percent gave a history of asthma in the family, while

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Studies X–XIII, Jour. Med. Research **36**: 231, 237, 243, 295, 1917.

Study XIV, ibid. **36**: 423, 1917.

Study XV, ibid. **37**: 51, 1917.

Studies XVI and XVII, Jour. Med. Res. [Boston] **37**: 277, 287, 1917.

Study XVIII, Jour. Am. Med. Assn., **70**: 897, 1918.

Study XIX, Jour. Med. Res. **40**: 229.

The cause and treatment of bronchial asthma, Med. Clinics of N. Amer., Boston, **1**: No. 4, 1918.

A clinical study of 400 patients with bronchial asthma, Boston M. and S. J. **179**: 288, 1918.

Treatment of bronchial asthma with proteins. Arch. Int. Med. **22**: 466, 1918.

The treatment of bronchial asthma with vaccines. Arch. Int. Med. **23**: 220–234. Feb., 1919.

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in the non-sensitive group, only 41 percent knew of the occurrence of asthma or hay-fever in the family. This proportion, 59 percent, of patients in the non-sensitive group with no known family history of asthma or hay-fever is probably unduly large, because the data are based almost entirely upon the statements of the patients themselves, often in answer to questions on several different occasions, and sometimes corroborated by other members of the family. Time and opportunity were lacking for interviewing members of the families and investigating the records of distant relatives, as would have been done, in accordance with the methods of C. B. DAVENPORT (1911), if a field-worker could have been assigned to the problem. Some of the patients were foreigners with very limited understanding of English. Others had come recently from distant parts of the country or had lost their parents in early childhood, with the result that they could give very little information concerning their family history. On the other hand, since there is no stigma attached to the occurrence of asthma or hay-fever in the family, the patients had no motive for concealment and their statements can probably be accepted as fairly truthful. The accuracy of the data is somewhat vitiated by the fact that other conditions, such as dyspnoea caused by disease of the heart or kidneys, and severe bronchitis in old age, often simulate bronchial asthma and are reported as asthma in the family history, thus occasionally giving a falsely positive family history. But with due allowance for error, the larger proportion of positive family histories among the patients who were sensitive to proteins seems too great to be accidental, and the conclusion may be drawn that asthma of the anaphylactic type has a greater tendency to run in families than does the non-sensitive asthma which approaches more nearly an intensified bronchitis. This result is in accord with the much more remarkable figures of Dr. F. M. RACKEMANN (1918), who found that "a history of either asthma, hay-fever, or food poisoning, in the immediate family, occurs in 58.7 percent of the cases of extraneous asthma, but in only 10.5 percent of the cases of intrinsic asthma." Roughly speaking, and allowing for differences in result from the fact that Dr. RACKEMANN uses the intradermal tests in the diagnosis of protein sensitization, whereas in this series of 400 cases the cutaneous method (WALKER and ADKINSON 1917) was used, which test gives a smaller proportion of positive cases, RACKEMANN'S "extrinsic asthma" corresponds to WALKER'S (1918 b) "sensitive group" and the former's "intrinsic asthma" to the latter's "non-sensitive asthma."

The group of 96 cases which were sensitive to proteins and which reported the occurrence of asthma in the family contains 48 males and

48 females. Twenty-nine patients began asthma before the age of five years, nineteen between five and ten years, nineteen between ten and twenty, twelve between twenty and thirty, twelve between thirty and forty, and five between forty and fifty. Thirty-eight inherited asthma directly from the mother or from the mother's family and thirty-nine from the father or the father's family, while in six cases asthma occurred in the families of both parents. Among the 96 cases with asthma in the family and who were not sensitive to proteins, eleven began asthma before the age of five years, thirteen between five and ten years, thirteen between ten and twenty, sixteen between twenty and thirty, twenty between thirty and forty, seventeen between forty and fifty, and five after fifty. This group presents a contrast to the sensitive 96 in that forty-two of the non-sensitive patients began asthma after thirty, while among the 96 sensitive patients only seventeen began asthma after thirty. This difference in the age of onset between sensitive and non-sensitive groups has been demonstrated by WALKER (1918 b), and is not affected in any way by the family history of the patient. Among the 96 non-sensitive cases there were 38 males and 58 females. In thirty-eight cases asthma occurred in the mother or the mother's family, in thirty-five cases in the father or the father's family, and in four cases asthma occurred in the families of both parents. In the whole series of patients who had asthma in the family, it occurred in the father's family in seventy cases, in the mother's family in seventy-two cases, and in the family of both parents in ten cases,while in forty cases a sister or brother was the only relative known to have asthma. Thus among both sensitive and non-sensitive cases with a known family history of asthma the disease occurs equally in the family of father and mother, and cannot definitely be said to be "inherited" more frequently from either parental side. Among the 85 cases which were sensitive to proteins but gave a negative family history, there were forty-eight males and thirty-seven females. Of this group of cases, nineteen began asthma before the age of five years, eleven between five and ten, twelve between ten and twenty, twenty between twenty and thirty, seventeen between thirty and forty, and six between forty and fifty. In the group of 103 non-sensitive patients with negative family histories, there were fifty males and fifty-three females. The age of onset was as follows:—under five, seven cases; between five and ten, seven cases; between ten and twenty, sixteen cases; between twenty and thirty, twenty-one cases; between thirty and forty, thirty-three cases; between forty and fifty, eleven cases; over fifty, seven cases. Here is shown the difference in age of onset between sensitive and non-sensitive groups already noted

among patients with a positive family history; among the 85 sensitive cases, twenty-three or about a fourth of the entire number began asthma after thirty, but among the 103 non-sensitive cases, 51 or one-half the cases began asthma after thirty. In both sensitive and non-sensitive groups, there is a large number of patients with an age of onset under thirty among patients with a positive family history, and more who began asthma after thirty among those with a negative family history. In the sensitive group, 85 percent of the cases with a known family history began asthma before thirty, while among those with a negative family history only 73 percent began asthma before thirty years. In the non-sensitive group, 55 percent of those with a known family history began asthma before thirty, while among those with a negative family history only 48 percent have an age of onset under thirty years. An earlier age of onset is found among patients where asthma is known to occur in the family.

Of the patients with a positive family history, the record in eleven cases was too incomplete for analysis and these must be discarded as unsatisfactory. The fathers of thirty-seven patients had asthma. In this group there were seventeen males and twenty females, nineteen patients were sensitive and eighteen were non-sensitive. Of the women seven were sensitive and thirteen not sensitive, and of the men, twelve were sensitive and five not sensitive. The mothers of nineteen patients had asthma. Of these nineteen patients, there were six men, only one sensitive, and thirteen women of whom four were sensitive. The fathers of two patients, a non-sensitive man and a sensitive woman, had hay-fever. In thirty-nine cases the father had asthma or hay-fever. The mothers of six patients, three males and three females, all sensitive, had hay-fever, and the mothers of two patients, a man and a woman, both non-sensitive, had bronchitis. Therefore twenty-five patients altogether gave a history of asthma or hay-fever in the mother. The sisters of seven patients, four men and three women, had asthma, and the sisters of three women, two of whom were sensitive, had hay-fever. The brothers of ten patients, six men and four women, had asthma, and the brothers of four patients had hay-fever. Four patients, two non-sensitive men and two sensitive women, whose parents did not have asthma or hay-fever, had paternal grandfathers suffering from asthma. Eleven patients, four sensitive and two non-sensitive males, three sensitive and two non-sensitive females, whose parents were normal, had maternal grandfathers with asthma. Four patients, two non-sensitive and one sensitive male, and one non-sensitive female, with normal parents, had paternal grandmothers with asthma. Eleven patients, one non-sensitive and three sensitive males, four sensitive and three non-sensitive females, whose parents were normal, had maternal grandmothers with asthma. Thus in eight cases the asthma while skipping the generation of the father, may have been inherited from the ancestors of the father. One non-sensitive boy, whose parents and grandparents were normal, had a paternal great-grandmother with asthma. In the families of eighteen other patients, asthma was reported collaterally in the father's family, but not in the direct line of inheritance. Twenty-two patients with normal parents may have inherited asthma from the maternal grandparents, the disease skipping a generation. In the families of fourteen other patients, asthma was reported collaterally in the mother's family, and in three other families hay-fever was reported. Taking the summary, sixty-six patients may have inherited asthma from the father's family, thirty-nine directly, eight skipping a generation, one skipping two generations, and eighteen collaterally. From the mother's family sixty-four patients may have inherited asthma, twenty-five directly, twenty-two skipping a generation, and seventeen collaterally. Thus taken altogether, the chance of inheriting asthma from the family of either parent is equal. The number of men and women inheriting asthma from the father is nearly equal, with a slightly larger number of women, but twice as many women as men inherit asthma from the maternal side, and although this proportion is less when the number inheriting hay-fever is added, the balance is heavier for the women. Altogether, thirty-six women inherit asthma directly from the parents, which is 25 percent larger than the number of men inheriting directly from the parents.

A comparison of sensitization between the groups with asthma in the family and those with a negative family history yields results as follows: In the first group eight patients were sensitive to horse alone; two to cat alone; four to bacteria alone; twenty-eight to pollens alone; two to wheat alone; one to feathers alone; one to wheat and potato; one to egg and wheat; one to the cereals, wheat, corn, rye, and rice; one to corn and egg; one to casein and potato; one to casein and wheat glutenin; one to casein, asparagus and the pollens; one to mackerel and beef; one to cod, halibut, and egg; one to oat and *Staphylococcus aureus;* one to green coffee and feathers; one to wheat, corn, casein, and ragweed; one to flaxseed and ragweed; one to feathers and tobacco; two to horse and egg; one to feathers and ragweed; one to horse, corn, potato, rice, red top, and timothy; one to feathers and wheat leucosin; twenty-five to horse and other animal hairs, pollens, food and bacteria; thus showing multiple sensitization in great variation. But the same condition and variety of sensitization is found in the second group, where the sensitization may be summarized as follows: To horse only, four patients; to cat only, two patients; to wheat only, four patients; to bacteria only, eleven patients; to pollens only, twenty-seven; to cereals, one; to egg, three; to wheat and other cereals, three; to chicken and salmon, one; to rye, rice, potato, and haddock, one; to dog, feathers, and casein, one; to horse and pollens, eight; to cattle and lobster, one; to cattle and pollens, one; to dog, feathers, and casein, one; to horse and pollens, eight; to bacteria and pollens, one; to boxwood and pollens, one; to horse hair and other animal hairs, food, bacteria and pollens, in various combinations, twelve. Hence there is no apparent distinction in kind, variety, or degree of sensitization, as demonstrated by cutaneous tests, between patients who give a family history of asthma and those who do not.

In the group of 85 sensitive cases with a negative family history, there occurred several patients whose remote ancestry and distant relatives were well known, with no other case of asthma in the family. In these cases the disease seemed to have developed as a sporadic phenomenon from apparently normal stock, with no previous intimation in the life of the individual or in the family history of a predisposition to asthma. One such case, C. S., (WALKER 1918 a) a girl of ten years, had eczema as an infant and at the age of two years began to develop asthma in attacks with running of the eyes and nose, wheezing and difficult breathing from hay-dust and tall grass and when she was near horses. Skin tests were positive with horse-dandruff protein and with timothy pollen, and after treatment with the proteins of horse hair it became possible for her to take riding lessons and to care for her pony without symptoms of asthma. During the course of treatment with timothy pollen, eczema developed upon her back and legs in an irritating form which seemed always worse just after an injection of timothy. When the timothy pollen treatment was stopped, the eczema disappeared. In this girl the cause of the asthma was sensitization to horse dandruff as was proved by the development of the symptoms of asthma when near horses, by the positive skin tests to the proteins of horse dandruff, and by the marked improvement of the patient when treated with horse-dandruff protein. The twin-sister of this girl, who resembled her closely in appearance, except that she was larger and more vigorous, had no eczema, asthma or hay-fever, and was not affected by horses or hay. In the family of six children, one older, and three younger than the twins, and in a large circle of cousins and other relatives, the patient was the only member of the entire family known to have asthma, eczema or hay-fever.

Another patient, M. D., (WALKER 1917) a woman, aged thirty-eight, who had suffered from severe asthmatic attacks for ten years, and who always developed a rash from contact with fur, or the hair of dogs and cats, gave positive skin tests with the proteins of cat hair. She recovered from her asthma under treatment with the proteins of cat hair, and after much treatment with cat-hair proteins she gave a nearly negative skin reaction to the cat-hair proteins. No other member of her family was known to have asthma.

F. L. D., a man of thirty-eight, developed asthma suddenly at the age of twenty-three, after a long ride on horse back, although he had ridden horses all his life with impunity. Skin tests with horse-dandruff proteins were positive. After treatment with horse-dandruff proteins the skin tests were much diminished in intensity, and the patient had no trouble when riding horses. This patient knew of no asthma among his sons, or among other relatives. These three cases are presented to illustrate the fact that from the presence or absence of asthma in the family history, it is not possible to draw any inference as to the cause, severity of the disease, or prognosis in any given patient with bronchial asthma.

From the standpoint of heredity, no clear distinction can be drawn between bronchial asthma of the sensitized or purely anaphylactic type as demonstrated by positive cutaneous tests with proteins, and a second or non-sensitive type, which, always giving negative skin reactions with the proteins tried, is more like an intensified bronchitis and is probably not due to anaphylaxis at all. These two types of asthma are distinct in clinical history, cause, treatment, and prognosis, but from the standpoint of heredity they seem to be equivalent and interchangeable. Like the anaphylactic type of asthma, the non-sensitive type seems to run in families, and almost invariably the two types of asthma do not run pure in the family histories but both types occur in the same family. Among the patients who have been tested with proteins, these two types of asthma occur among members of the same family without distinction or rule. Among asthmatic patients, the mother may be sensitive, the son not sensitive; the father not sensitive, the son sensitive; the mother sensitive, the daughter not sensitive; both parent and child sensitive, or both not sensitive; one sister sensitive, the other not sensitive; one parent sensitive and suffering from both hay-fever and asthma, the other parent sensitive and having hay-fever, the child not sensitive and not suffering from either hay-fever or asthma.

Not only must non-sensitive asthma be accepted as the equivalent of the anaphylactic type from the standpoint of heredity, but it is necessary also to accept as equivalents hay-fever, some urticarias, and occasionally eczemas caused by the ingestion of foods or other proteins, notably the eczemas in young children caused by eating eggs, milk or wheat. All of these symptoms indicate the existence of the anaphylactic state, i.e., sensitization to proteins. This point is well illustrated by the case, C. S., already presented, who in addition to asthma from horses had hay-fever from grass pollen. In the course of treatment with timothy pollen she developed an eczema which disappeared when the treatment was stopped. In cases sensitive to pollens the symptoms are more commonly hay-fever, but they may show asthma alone, or both hay-fever and asthma. Both hay-fever and pollen asthma are types of pollen sensitization, giving positive skin tests with the pollens and improving after treatment with the pollens to which they are sensitive. Among the patients sensitive to pollens, the mother may have hay-fever and asthma, the two sons hay-fever; the mother hay-fever, a son horse asthma, and an infant child an idiosyncracy for eggs; the mother horse asthma, the son fall hay-fever; the mother asthma and fall hay-fever, the son non-sensitive asthma; the father asthma and bronchitis, the son asthma; one brother asthma from foods, with bronchitis, the other brother fall hay-fever; one sister horse asthma and eczema and vomiting from eating eggs, another sister spring hay-fever, the niece asthma, the brother and aunt hay-fever; one sister with bronchitis and non-sensitive asthma although sensitive to wheat leucosin, the other sister sneezing when sifting wheat flour. Sometimes the sensitization to animal hair or to bacteria is manifested by sneezing and running of the nose rather than by asthma. Large doses of the proteins given in the course of treatment occasionally produced an immediate and temporary attack of asthma, and sometimes of hay-fever, urticaria, or eczema. From experiences like these, one is inclined to accept asthma, hay-fever, and sometimes urticaria and eczema, always coördinated by the skin tests or by the history and the symptoms, as different manifestations of the same anaphylactic state. The studies of family history were not extensive enough to show the occurrence of these other manifestations of sensitization to proteins in all the families where they may have been present, and with further study some apparently negative family histories might show the occurrence of some of these other manifestations. Although hay-fever, urticaria and eczema are accepted in this paper as the equivalent of anaphylactic asthma in the family history, all the patients included in the series of 400 cases had bronchial asthma. From the limitations of the investigation, and because asthma is sometimes caused by protein sensitization and sometimes not, this paper is restricted to the study of asthma as an inherited character, without attempting to solve the larger problem of the inheritance of sensitization to proteins in general.

In table 1 is presented a record of cases where two or more members of a family were tested with proteins. Thirteen instances are recorded in table 1 where the relationship was parent and child. In six of these both parent and child gave positive skin tests; in five, only one gave positive skin tests, while the other was non-sensitive; and in two, both gave negative reactions. Of the six pairs where both were sensitive, in only two did the parent and child react to the same protein, namely, ragweed pollen; the mothers, (M. F.) and (C. E. H.), had fall hay-fever with asthma, and in one case one son, (C. F.), in the other, two sons, (E. B. H. and W. H. K.), had fall hay-fever. Of the four sensitive pairs who did not react to the same proteins, in one the mother (G. H. I) who had spring hay-fever gave positive reactions with the pollens of red-top and timothy, whereas the son (F. I.) who had asthma was sensitive to horse. In another case the mother (J. G. W). with asthma was sensitive to horse and the son (J. M. W.), although he gave a positive reaction to cat hair, had only fall hay-fever and reacted positively to ragweed pollen. In one case the father (F. L. C.) with asthma in the spring and fall was sensitive to the pollens of golden-rod and ragweed, and the daughter (L. C.) with asthma and a loose cough was sensitive to egg and wheat bread, and was relieved by the omission of these foods from her diet. In another case the mother (R. L.) who had asthma and eczema was sensitive to horse and egg, whereas the daughter (B. L. S.) who had spring hay-fever was sensitive to red-top and timothy pollens. Of the group of five pairs where only one was sensitive, in one instance the father (W. B.) was sensitive, the son (J. B.) was non-sensitive; in two where the mothers, (A. G.) and (V. M.), were sensitive, the child, in one case the son (V. G.), in the other the daughter (E. M.), was not sensitive; in one instance both parents (M. L. P. and M. L. P.) were sensitive and the son (L. P.) not sensitive; and in another the father (H. D.) was not sensitive but the son (P. D.) gave positive skin tests with horse, cat, and dog. In two instances, (L. C. and R. C.) and (L. L. and V. N.), both mother and daughter gave negative reactions with all the proteins tried.

Among this group of thirteen pairs of relatives, in seven the child began asthma earlier than the parent, and the age of onset in the child was earlier by anywhere from six to thirty-five years than in the parent; in two instances the parent and child had approximately the same age of onset; in one case the age of onset in the mother was indefinite but probably later than that of her son; in two cases the daughter developed

TABLE 1

The sensitization of relatives as shown by cutaneous reactions to various proteins

(A) Parent and child, both sensitive

Name	Age	Age of onset	Sex	Relation	Symptoms	Sensitization	Family history
G. H. I.	35	28	F	Mother	Spring h.-f.*	Timothy, red-top	Infant daughter has urticaria from eggs
F. I.	7	8 mo.	M	Son	Asthma	Horse, bacteria	
J. G. W.	44	12	F	Mother	Asthma	Horse	Mother's sister had asthma
J. M. W.	14	12	M	Son	Fall h.-f.	Cat, ragweed	
M. F.	35	Chi.	F	Mother	Asthma, fall h.-f.	Ragweed, golden rod	None
C. F.	13	3	M	Son	Fall h.-f.	Ragweed, daisy	
C. E. H.	49	4	F	Mother	Fall h.-f. with asthma	Ragweed, daisy, red top, timothy	Of five sons, the second and fifth have hay-fever
E. B. H.	23	18	M	Son	Fall h.-f.	Ragweed, daisy	
W. K. H.	12	9	M	Son	Fall h.-f., asthma once	Ragweed	
R. L.	64	4	F	Mother	Asthma, eczema	Horse, egg	Daughter has hay-fever
B. L. S.	45	27	F	Daughter	Spring h.-f.	Red top, timothy	Sister has h.-f.
F. L. C.	32	Infancy	M	Father	Asthma, spring and fall	Ragweed, golden rod	None
L. C.	1½	Birth	F	Daughter	Asthma, loose cough	Egg, wheat	

(B) Parent and child, one sensitive

A. G.	30	20	F	Mother	Asthma, fall h.-f.	Ragweed, red top, timothy	Paternal uncle and aunt, and another uncle's three children have asthma
V. G.	12	6	M	Son	Asthma	None	
H. D.	67	40	M	Father	Asthma	None	Brother had asthma
P. D.	11	5	M	Son	Asthma	Horse, dog, cat	

* h.-f. = hay-fever.

TABLE 1 (Continued)

Name	Age	Age of onset	Sex	Relation	Symptoms	Sensitization	Family history
V. M.	50	13	F	Mother	Asthma, fall and winter	Ragweed	Mother had asthma
E. M.	16	7	F	Daughter	Asthma	None	
W. B.	39	33	M	Father	Summer asthma	Daisy, corn, clover	None
J. B.	16	14	M	Son	Frequent colds once wheezing	Red top, corn = slight	
M. L. P.	33	23	F	Mother	Spring h.-f., with asthma	Timothy, red top	None
M. L. P.	35	29	M	Father	Fall h.-f.	Ragweed	None
L. P.	9		M	Son	None	None	
(C) Parent and child, neither sensitive							
L. C.	42	20	F	Mother	Winter asthma	None	None
R. C.	16	Chi.	F	Daughter	Winter asthma	None	
L. L.	40	11	F	Mother	Winter asthma	None	Mother had asthma
V. N.	22	20	F	Daughter	Summer asthma	None	
(D) Aunt, nephew, and niece, all sensitive							
E. E. B.	47	31	F	Aunt	Fall h.-f.	Ragweed	Brother has asthma
R. B.	23	Chi.	M	Nephew	Fall and spring h.-f., asthma in childhood	Ragweed, golden rod, timothy	Mother, maternal grandmother had asthma
G. B.	17	1½	F	Niece	Asthma, eczema, bronchitis	Horse, ragweed, golden rod, wheat	Same
K. V. F.	46	39	F	Aunt	Fall h.-f., asthma	Ragweed, golden rod	None
R. G. V.	16	6	M	Nephew	Asthma	Ragweed, cattle, feathers, lobsters	Aunt has hay-fever
(E) Sibs, one sensitive; the other normal							
M. L. S.	31	12	F	Sister	Asthma, bronchitis	Oat, bacteria	Mother's mother, patient's brother had asthma
M. F.	14	Inf.	F	Sister	Frequent colds	None	Same

TABLE 1 (continued)

(F) Sibs, both sensitive

Name	Age	Age of onset	Sex	Relation	Symptoms	Sensitization	Family history
H. D. T.	16	6 mo.	F	Sister	Asthma, bronchitis	Dog, horse, cat, chicken, ragweed	Grandfather and father have asthma
C. T.	11	3	M	Brother	Asthma, eczema, bronchitis	Horse, ragweed, wheats = slight	Same
W. E. C.	31	24	M	Brother	Asthma	Ragweed, linden leaves, horse = slight	Father and two brothers have asthma
C. F. C.	25	10	M	Brother	Asthma	Horse, red top	Same
M. B.	13	Inf.	F	Sister	Asthma, eczema, vomiting from eggs	Horse, dog, egg, wheat	Mother has some hay-fever
J. O. B.	11	7	F	Sister	Spring h.-f.	Ragweed, golden rod, timothy, red top	Same
F. G.	42	22	F	Sister	Asthma summer and winter	Wheat leucosin	Father had asthma
H. M.	37	31	F	Sister	Hay-fever from wheat flour	Wheat, and other cereals	Same
W. M. O.	26	23	M	Brother	Asthma	Potato, casein	Maternal grandfather had asthma
F. O.	31	27	M	Brother	Hay-fever	Ragweed	Same
M. N.	46	Chi.	F	Sister	Asthma, hay-fever, eczema	Horse, ragweed	Mother had hay fever; brother had asthma
J. A. N.	36	Chi.	M	Brother	Hay-fever	Ragweed, timothy	Same

(G) Sibs, none sensitive

Name	Age	Age of onset	Sex	Relation	Symptoms	Sensitization	Family history
P. J. O.	48	31	M	Brother	Summer asthma	None	Brother has asthma
D. J. O.	33	21	M	Brother	Winter asthma	None	Same

asthma later than did the mother, in one case nine years later, and in the other thirteen years later than did the mother; and in one case where both parents were sensitive to pollens, the mother (M. L. P.) suffering from spring hay-fever with asthma and the father (M. L. P.) from fall hay-fever, the son (L. P.) has not yet shown symptoms of either disease and is non-sensitive, but he is still fourteen years younger than the earliest age of onset of his parents. It is interesting that this child was born before the father developed hay-fever and that the symptoms developed in the mother during pregnancy with this son. The failure of maternal transmission of sensitization in this case during pregnancy is contradicted by another case where the mother (G. H. I.) developed spring hay-fever during pregnancy and the son (F. I.) began to have asthma at eight months, but this mother and son do not have the same symptoms nor do they react to the same proteins. Therefore even in this case, which is the best example of the series of a condition of sensitization to proteins transmitted from mother to offspring during pregnancy, the sensitization if transmitted at all is not specific. Since in nine and probably in ten cases out of the thirteen, the age of onset in the child was the same or earlier than that in the parent, there may be some tendency for children of asthmatic parents, if they do develop asthma, to develop it earlier than did their parents.

Two family groups are presented, one including aunt and nephew, the other aunt, nephew, and niece, the latter two being brother and sister, all of whom happened to be sensitive to ragweed pollen. In the first family group, the aunt (K. V. F.), who was the father's sister, had fall hay-fever, and her nephew (R. G. V.), who began to have asthma thirty-three years younger than the aunt began to have hay-fever, was sensitive to cattle, feathers, and lobster, as well as to ragweed. The parents of this boy had neither asthma nor hay-fever. In the second family group, the aunt (E. E. B.) had fall hay-fever, the nephew (R. B.) had asthma as a child and later on he developed both spring and fall hay-fever at a much earlier age than did the aunt. The niece (G. B.) began to have asthma and eczema at eighteen months, and in addition to the fall pollens, she gave positive reactions to horse and wheat. The mother and the maternal grandmother of this brother and sister had asthma; the father seems to have no trouble, but in addition to the sister whom we have tested, he has a brother with spring hay-fever.

Of seven pairs of sibs tested, as recorded, two brothers (P. J. O. and D. J. O.), both with asthma, gave negative reactions to all the proteins tried. A brother and sister (H. D. T. and C. T.) both asthmatics, reacted alike to horse and ragweed. Two brothers (W. E. C. and C. F. C.), both with asthma, gave positive reactions to horse, one (C. F. C.) was strongly positive, the other (W. E. C.) only slightly, and both were sensitive to a pollen, one to ragweed, the other to red top. Of two sisters, one (F. G.) with asthma and bronchitis, happened to give a positive reaction to wheat leucosin, but she was not sensitive to the other proteins of wheat, neither did she improve when treated with wheat leucosin nor when wheat was omitted from her diet; the other (H. M.) who sneezed whenever she sifted wheat flour, gave positive reactions to wheat and other cereals but was as unresponsive as her sister to treatment with wheat and to the omission of wheat from her diet. Of a sister and brother, the sister (M. N.) who has asthma, hay-fever and eczema, is sensitive to horse and ragweed; the brother (J. A. N.) who has only hay-fever is sensitive to the pollens of timothy and ragweed. Of two sisters, one (M. B.) who had horse asthma, and eczema and vomiting whenever she ate eggs, was sensitive to horse, egg, and wheat, whereas her sister who had only spring hay-fever, reacted only to pollens. Of two brothers, one (W. M. O.) with asthma gave positive reactions to potato and casein, the other (F. O.) with fall hay-fever was sensitive to ragweed pollen. An eighth pair of sibs were tested, two sisters, of whom one (M. L. S.) with asthma and bronchitis was sensitive to oat and bacteria; the other with frequent colds but no asthma, gave negative reactions with all the proteins tried. But these two could be duplicated in almost any family in which a sensitive patient has a brother or sister, since in no family have we found every member suffering from hay-fever or asthma, and the normal individuals of course do not give positive skin reactions; hence this mixed pair, one sensitive and the other not, was not included in the series. Of the seven pairs of sibs presented, four pairs reacted alike to one or at the most two proteins; namely the brother and sister (H. D. T. and C. T.) both sensitive to horse and ragweed, two brothers (W. E. C. and C. F. C.) somewhat sensitive to horse, two sisters (F. G. and H. M.) who gave more or less adventitious reactions with wheat proteins, and a brother and sister (J. A. N. and M. N.) one with horse asthma, hay-fever, and eczema the other with fall hay-fever, both sensitive to ragweed. Two pairs, the sisters (M. B. and J. O. B.) and the brothers (W. M. O. and F. O.) where one had hay-fever and the other asthma, were both sensitive but not to the same pollens, and one pair (W. J. O. and P. J. O.) two brothers, were not sensitive.

Twenty-two family groups are presented altogether where more than one member has been tested on the skin with proteins. Of these twenty-two groups, in fourteen both members reacted to some protein; in five pairs, one member was sensitive to some protein, the other was not sensitive, and in three pairs, both members were not sensitive. Of the five mixed pairs, all were parent and child; in four the parent was sensitive, the child not sensitive, and in one the father was not sensitive when the son reacted to proteins. Of the four non-sensitive children of sensitive parents, in two cases the mother was sensitive, and in one case the son, in the other the daughter, was non-sensitive; once the son was not sensitive when the father was; and in the fourth case both parents were sensitive, while the young son was non-sensitive and was free from both asthma and hay-fever. Of the fourteen groups of relatives, all sensitive, six did not react to the same proteins, and eight did to some extent. Of the six not sensitive to the same proteins, four (G. H. I. and F. I.), (J. G. W. and J. M. W.), (F. L. C. and L. C.), and (R. L. and B. L. S.), were parents and children, two mothers and sons, one father and daughter, and one mother and daughter, and two were sibs, two sisters (M. B. and J. O. B.) and two brothers (W. M. O. and F. O.). Of these six pairs giving heterogeneous reactions, there were five pairs of which one member had hay-fever and the other asthma, the varying reactions corresponding to the different symptoms. In only one pair of these did both members suffer from asthma, and even in this pair, father and daughter (F. L. C. and L. C.), the reactions followed the symptoms and not the relationship, since the father had pollen asthma and the infant daughter had asthma from foods. Of the eight groups of relatives where all members were sensitive to the same protein, one pair was the two sisters sensitive to wheat leucosin where the symptoms did not altogether correspond to the skin reactions five groups included one of mother and son (M. F. and C. F.), one of mother and two sons (C. E. H., and E. B. H., and V. K. H.), an aunt and nephew (K. V. F. and R. G. V.), an aunt, nephew and niece (E. E. B., R. B., and G. B.), and one of brother and sister (M. N. and J. A. N.) where the element of pollen asthma and hay-fever explained the identical cutaneous reactions. The seventh pair were the brothers (W. E. C. and C. F. C.) somewhat sensitive to horse, but in neither brother was horse the fundamental cause of the asthma, and finally the brother and sister (H. B. T. and C. T.), both sensitive to horse and ragweed, but with many other individual factors which affect the asthma and the multiple sensitization. The evidence from this series of twenty-two tested groups of relatives, which included forty-seven individuals, of whom only thirty were included in the series of four hundred cases studied, indicates that sensitization to specific proteins is not transmitted from parent to child even when both have asthma and both are sensitive to foreign protein; that the child may be sensitive even when the parent is not; that the parent may be sensitive and that this sensitization is not necessarily transmitted to the child even when the asthma seems to be inherited. In other words, the cutaneous test is an index of the cause and symptoms of the individual case of bronchial asthma, but it bears no specific relation to the family history or to the problem of inheritance. When members of the same family have identical symptoms they may give identical skin reactions, but so may all other persons not related, who have the same identical trouble. For this reason, family histories and skin reactions in patients with hay-fever will correspond much more closely and seem to prove much more conclusively the existence of a specific inherited tendency than can any group of asthmatic patients. This series of relatives tested also lends support to the statement that sensitive and non-sensitive asthma, as well as hay-fever and some skin disturbances caused by the ingestion of proteins, must be accepted as equivalents from the standpoint of heredity. This detailed discussion shows also that in considering the behavior of bronchial asthma in inheritance the grouping into sensitive and non-sensitive types which is useful for diagnosis and treatment cannot be followed in studying family histories.

Many experiments have been performed on animals to discover the laws which govern anaphylactic shock in animals artificially sensitized, and the tendency has been to apply the reasoning by analogy from the results obtained in these experiments to the condition in patients suffering from asthma, hay-fever or other anaphylactic phenomena. It was the similarity between experimental anaphylactic shock in guinea-pigs and the asthmatic attack in the human which led MELTZER (1910) to advance the hypothesis that asthma is an anaphylactic phenomenon caused by sensitization to foreign protein. A brief review of the literature of animal experimentation will be given, in so far as it touches upon the problem of heredity. EHRLICH (1892), in studying the transmission in mice of immunity to certain substances, showed that female mice, immunized to ricin, abrin and robin, gave birth to young which possessed distinct resistance to these substances four weeks after birth, but that this resistance was lost by the beginning of the third month. This increased resistance was not transmitted to the grandchildren, and was transmitted only by the mothers. Immune fathers did not transmit immunity to their offspring. Normal offspring of untreated parents gained a considerable degree of immunity by nursing immune mothers. Later this study was extended to tetanus (EHRLICH and HUEBNER 1894), and it was shown that immune mother guinea-pigs and mice transmit immunity to young, but fathers do not, and the immunity transmitted to the offspring disappears in the second or third month. In guinea-pigs treated with diphtheria antitoxin by **Wernicke** (1895) and guinea-pigs and rabbits rendered immune to tetanus, anthrax, and cholera, by **Vaillard** (1896) it was found that the treated female always transmitted some resistance to her offspring, often to successive litters; the immunized father does not transmit immunity; the increased resistance in the offspring disappeared after the third or fourth month; experiments upon increased immunity through lactation were uncertain or negative; and except for one case of resistance in the second generation observed by **Vaillard**, no increased immunity was found among the grandchildren. **Theobald Smith** (1907) in an article on the transmission of passive immunity by immunized female guinea-pigs to their offspring says:

> "In a preliminary paper published several years ago, the writer called attention to the fact that certain female guinea-pigs under observation gave birth to young which had more than average resistance to diphtheria toxin. This increased resistance was observed in all litters until the death of the mother, and was nearly constant in amount for any given mother. . . . . The conclusions finally reached were that the manifestation of increased resistance in the young is due to the preliminary treatment of the mother with toxin-antitoxin mixtures, but the degree to which the mother reacts to treatment,—that is, the degree of passive immunity transmitted to the young,—is probably an individual or family factor. . . . . In no instance did the offspring of the treated resistant males possess any more than average or normal resistance. . . . . The transmission of increased resistance by the mother to her offspring is regarded by **Ehrlich**, **Wernicke** and others, as a passive process in the young. The immunizing antibodies are transmitted through the placenta *in utero*, and perhaps to some extent in the milk. This passive immunity is gradually lost with the increasing age of the offspring. This passive immunity is, therefore, of limited duration, most observers regarding it as lasting two or three months. . . . . . All observers . . . have found that the grandchildren of the immunized females have the usual resistance. . . . . **Vaillard** traced the persistence of active immunity in the immunized female through four litters of the same female. **Bulloch** found traces of hemolysins in an immunized rabbit three hundred and eighty-seven days after innoculation. . . . . The writer has records of a considerable number of guinea-pigs which transmitted immunity to their offspring for over a year. . . . . Though the male parent does not transmit directly any passive immunity, there is no evidence to show that he does not equally with the mother transmit the capacity for producing antibodies, which capacity varies much from family to family, quite independently of the treatment."

Rosenáu and **Anderson** (1907) clearly separate the anaphylactic reaction to horse serum and the immunity to diphtheria produced by the injection of diphtheria antitoxin.

> "In our previous work we showed that hypersusceptibility to the toxic effects of horse serum may be transmitted from the mother guinea-pig to her young. Later one of us (ANDERSON) showed that the female guinea-pig may transmit hypersusceptibility to horse serum and immunity to diphtheria toxine at the same time. . . . . Our present studies corroborate the fact that hypersusceptibility to the toxic action of horse serum is always transmitted from the mother guinea-pig to her young. This function is solely maternal; the male takes no part whatever in the transmission of these acquired properties. Whether this maternal transmission is hereditary or congenital cannot be definitely stated. We are able to exclude milk as a factor in transmitting the hypersusceptibility to the toxic action of horse serum, by a series of exchange experiments. 'Exchange' experiments consist in at once placing guinea-pigs born of a susceptible mother to nurse with an untreated female and, in exchange, the young of the untreated female are at the same time placed to nurse with the susceptible female. From these 'exchange' experiments we learn that hypersusceptibility is not transmitted from the young through the milk. We also learn from our experiments that hypersusceptibility may be transmitted from mother to young whether the mother is sensitized before or after conception. The fact that this influence may take place after conception might be taken to indicate that the condition is congenital and not hereditary. . . . . We have never seen symptoms resulting from the first injection of horse serum in the guinea-pig born of an untreated mother."

GAY and SOUTHARD (1907) show conclusively that this transmitted hypersusceptibility to horse serum is passive artificial sensitization.

> "There is further evidence that guinea-pigs refractory to horse serum still contain the sensitizing substance of the serum unneutralized. The offspring of refractory animals, born at a period when the mother is resistant to the toxic action of horse serum, are sensitive, not resistant."

Guinea-pigs immunized by repeated doses of horse serum, bore young which when injected at ages varying from six to nine weeks, with much smaller doses of horse serum than the mother had received before the birth of the young, without ill effects, had typical anaphylactic shock, and showed the pathological findings produced by that condition.

> "There is an analogy between these last experiments on the transmission of sensitivity of offspring from refractory mothers and certain experiments of ROSENAU and ANDERSON, and of ANDERSON; these authors found that the young of sensitized guinea-pigs were also sensitive to horse serum. It was only a step further for us to demonstrate that normal pigs may be rendered sensitive to horse serum by injecting them with the serum of sensitive pigs and awaiting the proper incubation."

VAUGHAN (1913) says:

> "The transfer of the condition of sensitization from the mother to her offspring is an illustration of homologous passive anaphylaxis. This has been studied especially by ROSENAU and ANDERSON, GAY and SOUTHARD, and OTTO. The latter has found the young sensitive at forty-four hours after birth." . . . .

The first injection of the foreign protein is without manifest effect, but in reality it has a most profound effect. It induces changes which may continue throughout life and may be transmitted from mother to offspring."

In experimental anaphylaxis GROER and KASSOWITZ (1914) showed that the diphtheria antitoxin demonstrated by SCHICK to be present in blood and tissues of some human beings, were transmitted passively from the mother to infant through the placenta. VON DUNGERN and HIRSCHFIELD (1911) have claimed that iso-agglutenins are inherited, often according to Mendelian law.

Passive artificial sensitization transmitted from the treated female guinea-pig to her young, behaves as a different phenomenon from bronchial asthma in man. The artificial condition is transmitted only from the treated mother to her offspring, whereas asthma occurs with equal frequency in the families of either parent. The young of an artificially sensitized animal are most sensitive soon after birth, and the sensitization is lost within a few months, whereas in the human, asthma, or sensitization to proteins may be manifested in very young infants, begins most frequently in children under five years, but it may be shown for the first time at any age under thirty. The successive litters of a sensitized guinea-pig are all equally sensitive, whereas of the five sons of a woman who has hay-fever, the second and fifth may have hay-fever, while the first, third, and fourth sons are not affected by pollens; that is, normal children may be born between and following sensitive ones. The sensitization in animals is never transmitted by the offspring of treated mothers to the third generation, except possibly in one case observed by VAILLARD whereas asthma or sensitiveness to proteins may be manifested in man through three or even four successive generations. In asthma the condition may be present in grandparent and grandchild, but not manifested in the parent, thus skipping one and sometimes two generations, or the asthma or protein sensitization may be present in aunt or uncle and nephew or niece, while the parents are normal. In animals the young of untreated females never react without a previous sensitizing dose, but according to TALBOT, infants may react to the very first cow's milk or egg with which they are fed, and in four infants a positive skin test to egg was obtained before egg had ever been eaten, but there is no positive proof that the mothers of these infants were sensitive to egg. In the two cases of the present series, where the mother developed first symptoms of sensitization to pollens during pregnancy, one child is normal, and the child of the other mother developed asthma when eight months old. Moreover, the sensitization in the young of treated females is always for the specific protein with which the mother was treated, and the anaphylactic shock produced by the first injection into these sensitized young of the protein to which they are sensitive differs in no respect from the anaphylactic shock which might be produced in the mother or in a normal pig transfused with blood from the mother. Sensitiveness to foreign protein in the human, if it is inherited at all, is almost always not specific, either as to the particular protein, or as to the symptoms produced. The evidence from family histories of asthmatics is against inheritance of specific sensitization. All the evidence indicates therefore, that bronchial asthma is not maternal transmission conveyed from mother to child through placenta or milk, not congenital, caused by any action during pregnancy upon the cells of the foetus that produce sensitization, but if it is inherited at all, it behaves as a character transmitted equally by the germ-plasm of either parent, independent of any manifestation of asthma or sensitiveness to protein in the somatic cells of the parent. The nearest approach to this condition in animals is noted in the quotation from THEOBALD SMITH, that some families of guinea-pigs vary much in their ability to develop antibodies to diphtheria toxin.

Throughout the literature which deals with bronchial asthma as an anaphylactic phenomenon, suggestions and statistics as to the behavior of the disease in inheritance are given by many authors. S. J. MELTZER (1910), one of the first to interpret asthma by the phenomena of anaphylaxis, says:

> "Heredity also seems to be an influential factor in some cases . . . . . The sensitizing effect may be transmitted from mother to offspring, which however may gradually dwindle away. . . . . The sensitization to anaphylaxis may be hereditary or acquired; so is the disposition to asthma either hereditary or acquired."

EDWARD LESNÉ and CHARLES RICHET fils (1913), discuss the same problem (translation by the present writer):

> "Anaphylaxis caused by eggs is found sometimes among many members of the same family; hence there seems to be a hereditary predisposition to it. An interesting case is that of GELPKE, who has observed in mother and child an absolute intolerance to eggs. One of us has published, with LAROCHE and SAINT GIRONS, an observation which shows through four generations the existence of an anaphylaxis to eggs: the great-grandfather, born in 1775, was afflicted with an absolute intolerance for eggs and cream; this intolerance became proverbial in the family who have kept it in mind; the grandfather, born in 1807, presented an anaphylaxis much less marked; he however could not eat the slightest bit of meringue without being sick. Of his four children, the first is normal, two others are intolerant of eggs and cream and look upon egg as a poison; the fourth was intolerant of eggs until the age of twenty-five years, since then his

intolerance has diminished. Of the three present grandchildren, one is unaffected, the other two are intolerant, the one lightly, the second strongly. This case is comparable to the cases of anaphylaxis to milk which occur among the children of a single family."

K. K. KOESSLER (1913) reports three cases of asthma caused by eggs, two with no family history of asthma, and a third patient, a woman aged twenty-eight, whose father was subject to hay-fever and had an intolerance for eggs in childhood, had asthma from eating eggs and asparagus.

"The little daughter of the patient, five years old, eats eggs without ill effects. . . . . In our cases . . . . the first sensitizing dose seems not to have been given. But this is not essential. For the substances which split the protein in the blood to toxic products may be present from birth in an abnormally high amount, through a peculiar diathesis of the individual. . . . . Anaphylaxis is a condition which may be inherited, so is asthma. . . . . The identification of asthma as a manifestation of an inherited or acquired allergy or anaphylaxis is able to unite and explain in a satisfactory manner the heterogeneous theories of the underlying mechanism of the asthmatic crisis."

RICH (1914), presenting cases of infantile sensitization to egg albumen, observes:

"One of the most interesting features of this disorder is the frequency with which it appears in a family. In five of my ten cases there was good evidence of this disorder in the previous generation. In two the mother, once the father's sister, once the father's brother, once the mother's brother. VAUGHAN also found that experimental sensitization to protein may be transmitted to the young."

FRITZ B. TALBOT (1914) reports six cases of "egg poisoning," two with no family history, four with a family history of some anaphylactic phenomenon, but none with a history of specific sensitization to egg, except that the younger brother of one patient had some idiosyncrasy to egg. Three times the father had hay-fever, and twice the son, in addition to intolerance to egg, had hay-fever.

"The family history suggests that a sensitive condition of the blood may be transmitted from one individual to another. . . . . If this is not inherited directly, a predisposition toward sensitization may be inherited. It seems to be more probable that the sensitivity may be acquired."

HYMAN ISKOWITZ (1915) says:

"Asthma may be hereditary or acquired. A large percentage of cases of bronchial asthma have either a hereditary history or a history of protein sensitization. . . . . A predisposition to the condition is nearly always present."

Oppenheimer and Gottlieb (1915), in an article on hay-fever, speak of inheritance:

> "In all likelihood, there exists in the patient an individual susceptibility to this particular disease, which seems to have some relation to heredity, for this and other allied ailments are frequent in given families. Among our patients there are two brothers with hay-fever, a brother and sister with hay-fever; a woman with hay-fever whose son suffers from asthma; two cases in which a father and one or more of his children suffer from hay-fever; a young woman with hay-fever, who had intense eczema as a child and whose mother suffers with eczema that is rebellious to treatment."

The same authors, writing on the subject a year later (Oppenheimer and Gottlieb 1916) are even more impressed with the tendency of hay-fever to be inherited.

> "Heredity plays a very important rôle in the etiology of pollen disease, as we have shown that over 90 percent of our patients have other members of their families who suffer with allied complaints. . . . . Any denuded surface of the body is a suitable place for the parenteral absorption of the proteins of pollen, so that given a patient whose antecedents, either in collateral or direct line, have shown themselves to be sensitive to any of the above mentioned substances, that patient if so exposed, will in all likelihood develop pollen anaphylaxis."

F. B. Talbot (1916), in a second article on asthma in children, says:

> "The histories of most of the patients studied have shown that there is a hereditary predisposition to allergy; that the parents or close relatives of the children have asthma, hay-fever, or an idiosyncrasy to some food. During the year 1914–15 the writer made a routine skin test for egg albumin on all of the children and infants admitted to the Children's Ward at the Massachusetts General Hospital, and out of 85 admissions, three infants gave a positive skin test to egg albumen. They were respectively, three, four, and eight months of age. The mothers of the infants on being carefully questioned, said that the babies had never eaten egg in any form. A fourth case, in private practice, aged fourteen months, which had never eaten egg, gave a positive skin test to egg white. Schloss and Worthen report the same results in two infants, and Blackfan in one nine weeks old. It seems certain therefore, that these infants had a hereditary and not an acquired sensitization to egg albumen."

Later in the same article, Dr. Talbot continues:

> "A family history of asthma, hay-fever, rose colds, eczema, or idiosyncrasy to some food, was present in 19 out of the 23 cases, while in the remaining four cases, there were no notes in the family history on these points. Berkhead also found a 'hereditary element' strongly marked in 16 percent of his cases. It seems therefore that there is a strong family predisposition to asthma."

In an article on idiosyncrasy to cow's milk, the same author (Talbot 1916 b) says:

"Certain individuals whose parents and close relations give a history of such anaphylactic phenomena as asthma, hay-fever, chronic urticaria, or idiosyncrasies to foods, have a hereditary predisposition to sensitization. . . . . In other instances the first cow's milk, and all subsequent bottles that are given to the baby are vomited immediately, in which case we must assume that sensitization was hereditary, and present at birth."

In discussing the third paper of TALBOT on "Asthma in childhood III," ALEXANDER C. HOWE (1917) spoke of the bearing of the family history on the individual case:

"These subjects that are sensitized have an inherited taint. You will never find a case of hay-fever or asthma in a child but you will find that its ancestors had hay-fever or asthma, or acute gastric attacks from eating certain articles of food, or hives, or some other form of anaphylaxis. . . . . That is to say, if there is a decided history of sensitization in both parents, the child will frequently in a large percentage of cases develop some form of anaphylaxis by the fifth year. If there is a strong taint in only one side of the family they will surely develop it before the tenth or fifteenth year, and when the taint is only a slight one on one side, and there is none on the other, it may run to the twentieth or twenty-fifth year. By taking these facts into consideration, you can frequently determine by the history when it is possible for the subject to become sensitized—and to protect them accordingly."

FRANCIS M. RACKEMANN (1918), reporting on one hundred and fifty cases of bronchial asthma, says:

"The family history is here very interesting and important. There was a history of either asthma, hay-fever, hives, or violent poisoning from food in forty-four of this group of patients; twenty-seven on the mother's side and two on both; not stated in thirty-three. . . . . From this we may say that a positive family history indicates the probability of a particular case of asthma belonging to the extrinsic rather than the intrinsic group."

WARFIELD T. LONGCOPE (1915) in his Harvey Lecture on "*The susceptibility of man to foreign proteins*," discusses the problem of heredity at length, from both the experimental and theoretical aspects:

"The history of idiosyncrasies in certain families, such as the tendency to asthma or hay-fever or the susceptibility to certain foods, has long been recognized as very common. . . . . Hypothetically this may take place in one of three different ways: (1) as true inheritance through the germ-plasm of the cells, either of the father or mother; (2) by direct influence of the immunizing agent that affects the mother, upon the cells of the foetus which produce active immunity in the child; (3) by passive transference of the immune bodies from the mother to foetus by way of the blood or milk. . . . . The facts so far collected, regarding the familial tendency of idiosyncrasy to foreign protein do not accord absolutely with those found in experimental immunity and anaphylaxis from mother to offspring. In the first place, sensitization in man is not transient, but often of years' duration. In the second place it may occur through

> four generations, and in the third place, as occurred in the extraordinary family described by **Laroche**, **Richet** and **St. Girons**, in the male members. And finally the sensitization may not always be to the same protein. In at least one family which we have studied, the father was sensitive to horse serum and the son to egg white. If inheritance is a factor, therefore, it cannot be by means of passive transfer from mother to infant, but in some instances at least may be a true inheritance of cell characteristics derived either from the father or mother. The whole problem, since it is one of greatest importance, needs careful study, but one is inclined to suggest that occasionally sensitization towards foreign protein may be an inherited characteristic of the germ-plasm and often not highly specific in character. . . . . The high degree of susceptibility in some people, the multiplicity or lack of specificity of sensitization, and the distinct tendency for it to occur in families differentiate these individuals from the artificially sensitized and suggest that there is some unknown factor here which is absent in men and animals subjected to artificial sensitization."

Robert A. Cooke and **Albert Vander Veer** (1916), give data upon 621 cases of sensitization:

> "The antecedent, direct, or collateral history was negative in 260, positive on one side in 205, positive on both sides in 39, and discarded as incomplete in 117. . . . . Of 504 cases with satisfactory history there was positive antecedent, direct or collateral history in 48.4 percent. . . . . Inheritance therefore does exert a distinct effect upon the age of onset of symptoms of sensitization; the more complete the inheritance the earlier the manifestation. . . . . The offspring of a sensitive parent are not born sensitive. . . . A parent may transmit a tendency to sensitization without himself being sensitized. . . . . That placental sensitization is not an important factor is evidenced by the fact that the inherited character is as frequently paternal as maternal and that in cases where the inheritance has been maternal, the clinical form in the child is much more apt to be different from the mother (45.4 percent) than it is to be identical (18.6 percent) and is no more apt to be identical with the mother than with the father. . . . . We must say that the results of a clinical study compel us to conclude that sensitized individuals transmit to their offspring not their own specific sensitization but an unusual capacity for developing bioplastic reactivities to any foreign proteins."

Analyzing their data statistically the authors conclude that this capacity behaves as a dominant Mendelian character.

Before attempting an analysis of individual family histories for evidence that bronchial asthma is inherited according to **Mendel**'s law, a brief summary of the fundamental conceptions of heredity is in order. According to **Weismann**'s doctrine of the continuity of the germ-plasm, race characteristics are transmitted in a continuous stream of material from the germ cell of the parent to that of the child, independent of the environment or of the manifestation of inherited characteristics in the somatic cells of the individual body, which from the standpoint of heredity is only the vehicle of the germ cells. From the fact that during the maturation of the germ cells, before the union of egg and sperm to form the first cell of the new individual, the number of chromosomes is reduced to one-half the somatic number, so that, before their union, egg and sperm cells or gametes possess half the nuclear content of the normal soma cell, arose the conception that each inherited character has its material origin or basis in determiners present in the chromosomes of the germ cells; that these determiners are paired until the reduction division, but single in the mature egg and sperm. In the zygote which results from the fusion of egg and sperm—the first cell of the new individual, from which develop both soma and germ cells of the offspring—these determiners are again paired, but one member of each pair is derived from the egg, the other member from the sperm. Hence half the inheritance comes from either parent. If in any particular pair of determiners, the inheritance from the two parents is exactly the same, the child is said to be pure or homozygous with respect to that single character, but if the determiner from one parent differs from that derived from the other parent, the child is hybrid or heterozygous with regard to that character. If in the germ cells only one determiner is present, that is, the individual is heterozygous, the person is said to be simplex with regard to that character, but when the individual is homozygous or pure, and consequently bears in the germ cells two determiners for the character, he is said to be in the duplex condition. According to the laws formulated by MENDEL, and substantiated by DE VRIES, CORRENS, TSCHERMAK, and many others, the characteristics inherited from the two parents do not blend, but are segregated unmixed in the germ-plasm and are so transmitted. The offspring resembles one parent or the other with regard to any given character, but he is capable of transmitting to his offspring the characters inherited from both parents. A character which prevails in the soma cells whenever it is present in the germ cells is called a dominant character, while a character which may be present in the germ cells without manifesting itself in the soma cells is said to be recessive. Thus people who show a dominant character, may be pure dominant and capable of transmitting only the dominant character to their offspring, or they may be heterozygous with respect to that particular character. In the latter case, the hybrid individual has germ-plasm that is simplex with respect to the dominant and its corresponding recessive, and the germ cells which he produces will be of two sorts, one bearing the dominant character alone, and the other the recessive, according to which of the two determiners happens to be eliminated in the reduction division of the germ cell. Theoretically these two kinds of germ cells are produced in equal numbers; hence half the offspring of a hybrid individual mated to a recessive, would show the dominant character, and the other half the recessive. The recessive character, when present in the germ-plasm of a hybrid or heterozygous individual is not manifested in the soma cells, but is transmitted, as has been said, by one-half the germ cells. When only the recessive determiner is present in the fertilized egg (zygote) the individual is duplex (homozygous or pure) with respect to that character, and the soma cells manifest the recessive character, and all the germ cells produced from that zygote bear the recessive character.

On the theory that inherited characters are either dominant or recessive, and that a homozygous individual bears germ-plasm in which the determiners for any given character are alike, hence duplex, while in a heterozygous individual the germ-plasm is simplex, there are six possible types of mating, which may be represented diagrammatically as follows: *D* stands for a determiner of any dominant character and *R* for the corresponding recessive, the two together standing for the gametic character of parent and offspring.

Mating	Parents	Offspring
1	*DD* × *DD*	All *DD*; all showing the dominant character and transmitting it only.
2	*DD* × *DR*	*DD* + *DR*; all show the dominant character, but only one-half are duplex, and the half which are simplex are capable of transmitting the recessive character to one-half their offspring.
3	*DD* × *RR*	All *DR*; all show the dominant character, but are capable of transmitting the recessive to half their offspring.
4	*DR* × *DR*	*DD* + 2*DR* + *RR*; three-fourths of the offspring show the dominant character; one-fourth are duplex or homozygous, and can transmit only the dominant character; one-half are heterozygous dominant, transmitting the dominant character to one-half, the recessive character to the other half of their offspring; one-fourth show the recessive character, and transmit only the recessive character to their offspring.
5	*DR* × *RR*	*DR* + *RR*; half are pure recessive, and half, while showing the dominant character, will transmit it to half their offspring.
6	*RR* × *RR*	All *RR*.

If bronchial asthma is inherited in the manner of a Mendelian trait, the disease is probably either dominant or recessive to the normal or non-asthmatic condition. If the asthmatic condition is dominant over the normal, it will show as a somatic character wherever it is present in the germ-plasm, whether the individual is a pure dominant, that is, inherits asthma from both father and mother, or whether he is a heterozygous dominant, inheriting asthma from only one parent, and capable of transmitting the normal condition received from the other parent to one-half his offspring. If the asthmatic condition is dominant, then any normal individual would be a homozygous recessive, and not capable of transmitting the asthmatic character. On the other hand, if bronchial asthma is recessive to the normal condition, all asthmatic individuals must have inherited the character from both parents and are capable of transmitting the condition to all their offspring. Normal individuals by this hypothesis would be of two sorts, homozygous normals, not capable of transmitting asthma, and heterozygous normals, who, while not having the disease, have inherited it from one side of the family, bear it recessive in their germplasm, and are capable of transmitting it to one-half their offspring.

⊡ = ♂ normal male.
○ = ♀ normal female.
■ ● = asthmatic male and female.
◨ ◑ = male and female with hay-fever.
▢ ◯ = male and female with other asthmatic tendencies.
🄴 Ⓔ = male and female with eczema.
m = miscarriage.

KEY TO SYMBOLS

By this hypothesis, it would be possible for two apparently normal parents, if both carried the asthmatic character in the simplex condition, to bear asthmatic children.

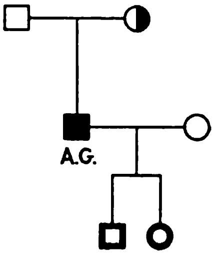

FIGURE 1

To determine whether bronchial asthma behaves as a Mendelian character in heredity, it is necessary to examine individual family histories in which asthma and allied conditions occur. In the G. family, there is evidence of protein sensitization in three generations. The mother of the patient has had hay-fever for many summers; the father of the patient was normal. The patient, A. G., a physician, aged 28, has had hay-fever for seven summers, and similar symptoms whenever he goes near horses or guinea-pigs, and with the hay-fever and with changes of weather he sometimes has asthma. By the cutaneous tests, he is sensitive to horse, guinea-pig, pollens of the grass and composite families, and various bacteria. Following injection of pollen he had urticaria, sneezing, choking and marked constitutional symptoms. His two children, a boy and a girl, aged eight and five years respectively, both have urticaria after eating strawberries and lobster. The wife of the patient, of unknown family history, is normal. In this family history, the asthma seems to behave like a Mendelian trait, dominant over the normal condition.

In the N. family, the asthmatic condition appears twice in three generations, the victims in each case being the daughter of a normal father and an asthmatic mother. In the fourth generation are three young children who as yet have not developed asthma. There is no evidence (both the patient and her mother were tested with many proteins) in this family, of sensitization to proteins. In this family, the asthmatic condition seems to behave like a character dominant over the normal conditions, and in this particular case, to be transmitted from mother to daughter.

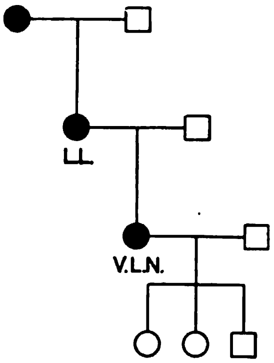

FIGURE 2

In the M. family occurs an almost identical family history, in that for two generations the daughter seems to inherit asthma from an asthmatic mother and a normal father. The mother of the patient, V. M., a woman aged 50, had asthma, but the older brother and sister were normal, her daughter, aged 16, has asthma, but three younger children, two girls and a boy, are normal. The mother is sensitive to ragweed pollen, the daughter was negative to all the proteins tried. Here again the asthmatic condition seems to be dominant over the normal, and to be transmitted from mother to daughter. The maternal grandmother, however, had a son and daughter who did not show the asthmatic condition, this could be explained by the hypothesis that the asthmatic grandmother was heterozygous with respect to the asthmatic character. The mating then would be like type 5, where half the offspring theoretically are pure recessive (normal if asthma is dominant), and half, while showing the asthmatic character, would be heterozygous and transmit the normal condition to half their offspring. The patient, V. M., must be of this heterozygous type; of her four children, one is asthmatic and three are normal.

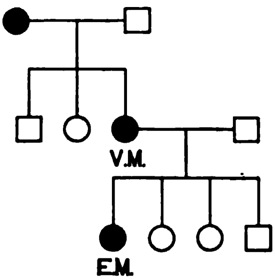

FIGURE 3

In the S. family the father of the patient had asthma; the mother, six sisters and one brother are normal; six other sibs died in infancy. The husband of the patient is normal, and none of her six young children have developed asthma as yet. The patient was sensitive to wheat, potato, and bacteria. Here asthma inherited from the paternal side seems to be dominant in the patient over the normal condition found in the mother; the seven normal children of the same parents could be explained, as in the M. family, by the hypothesis that the father was heterozygous and transmitted the normal condition to his other children; the theoretical number would be half the offspring normal and half asthmatic. This family history could also be interpreted by the opposite hypothesis, that asthma is recessive to the normal condition; the normal condition inherited from the mother is dominant over the asthmatic condition inherited from the father, in seven of the eight children, but these seven normal children are all heterozygous, and bear the asthmatic condition as a simplex character in their germ-plasm; the father, who must carry the asthmatic character in the duplex condition, if asthma is recessive, transmitted asthma to the patient, as to all his other children, and in addition, if the patient received the asthmatic character from the mother, who might be heterozygous, and hence capable of transmitting asthma, she would be a pure recessive, and hence asthmatic like her father. Marrying a normal man, the asthmatic condition would be transmitted by the patient to all her children, but none of them would have asthma because it would be in them recessive to the normal condition inherited from the father.

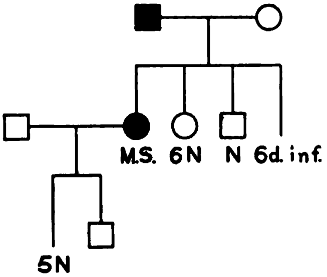

FIGURE 4

In the D. family the patient and his brother both had asthma of the non-sensitive type; of the eighteen children of the patient, by two mothers, all are normal except the youngest, a boy who has asthma and is sensitive to cat. If asthma is a dominant character, it was transmitted by the patient only once out of a theoretical nine times. If the asthmatic condition is recessive to the normal, and the first wife were a duplex normal, the ten children of the first family would all be normal; if the second wife were a simplex normal, she would be expected

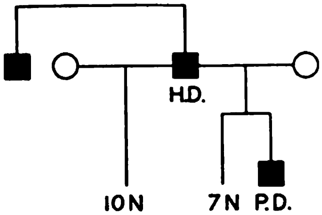

FIGURE 5

to transmit asthma to half her offspring; hence, if the patient, H. D., is a pure recessive, half the children of the second marriage would be expected to develop asthma; one out of a theoretical four is asthmatic. This last interpretation brings the theoretical expectation nearest the actual facts.

In the C. family, the grandparents of the patient are unknown. The father had asthma all his life, but after scarlet fever at the age of sixty the disease left him. Of the eight children of this asthmatic father and normal mother, four had asthma and four were normal. The patient, M. A. C., a woman, aged 56, the oldest of the family, suffering from asthma and diabetes, not sensitive to proteins, began to have asthma when she was a child, following scarlet fever. Her sister, three years younger, developed asthma after a cold contracted during scarlet fever when she was eighteen or nineteen years old. The brother began to have asthma following a cold contracted during measles, and he died from dropsy at the age of twenty-four. The youngest child, a girl, who had asthma, died from "heart failure" at the age of thirty. Two normal sisters are living and well. A brother who did not have asthma died from pneumonia. None of these eight children had offspring, except the second daughter who married a normal man and whose seven children as yet give no evidence of asthma. By the theory that asthma is dominant to the normal condition, if the father were a simplex asthmatic, half of the offspring would be expected to be asthmatic and half normal, which is exactly what happened. On the theory, that asthma is recessive to the normal condition, and an individual in order to show asthma must bear the tendency in the duplex condition, if the mother of the patient were a duplex normal, all the children would have been normal, but capable of transmitting asthma. But since half the children had asthma, it is possible that the mother was a simplex normal, that is not asthmatic but capable of transmitting asthma. In this case half the children would inherit asthma from both the father and mother, and would be asthmatic, while

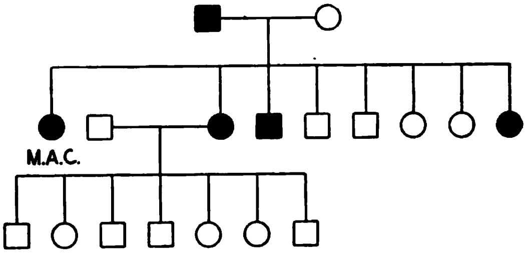

FIGURE 6

the other half would be normal like the mother but capable of transmitting asthma. The asthmatic daughter who married the normal man, would be expected to transmit asthma to half her offspring, if asthma behaves as a dominant character; but if asthma is a recessive, and her husband was a duplex normal, all her children would be simplex normal. From the family history, it is difficult to render a decision in favor of either theory, but the fact that none of the children of the second daughter have asthma is in favor of the doctrine that asthma behaves as a recessive trait.

In the G. family, the history differs from the group which has just been discussed, in that the parents of the patient were both normal. The family history of the mother is unknown. A sister and a brother of the father had asthma, and the three children of a normal brother of the father also had asthma, so these first-cousins of the patient also present the phenomenon of asthmatic children born of normal parents. The patient, a woman, aged 30, has suffered from asthma for ten years both winter and summer, and is sensitive to pollens and bacteria. Her husband and young son and daughter are normal; her oldest child, a boy of twelve years, has suffered from asthma for six years and is not sensitive to proteins. By the theory that asthma is a dominant trait, and hence present as a characteristic of every individual who is capable of transmitting the condition, it is not possible to explain how the children of normal parents can inherit a tendency to asthma which runs in the family and is manifested in grandparents, aunts or uncles, cousins or more distant relatives. By the theory that asthma is recessive to the normal condition, this skipping of a generation can be explained; but by that theory, every asthmatic individual must be duplex with regard to that character, that is, must have inherited the asthmatic tendency from both parents; and where both parents are

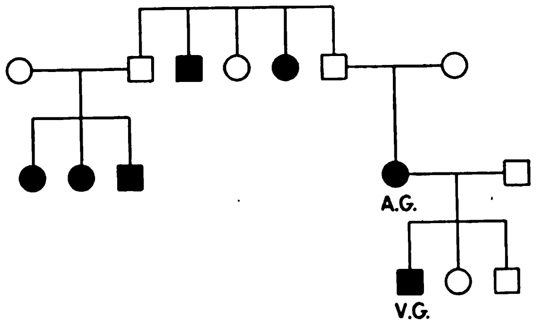

FIGURE 7

normal this is difficult of demonstration, and sometimes seems absurd. That asthma occurred in the family of the father of the patient is manifest; and with an asthmatic brother and sister, it is not improbable that the father was a simplex normal, that is, capable of transmitting the trait to half his offspring. But there is no evidence that the mother of the patient was a simplex normal, who transmitted the asthmatic tendency to her daughter, the patient; yet this assumption seems to be necessary to an interpretation of this family history by the Mendelian law. Moreover it is necessary also to assume without evidence that the husband of the patient is a simplex normal transmitting asthma to half his offspring, in order to explain the asthma in the son.

In the E. family the patient, a man, aged 25, who had eczema in childhood, has had asthma for twenty years, and by the cutaneous test is sensitive to animal hairs, foods, and pollens. The youngest of a family of five, whose sister, three brothers, and parents are normal, the patient seems to inherit asthma from his paternal grandmother. The father who is sometimes short of breath but does not have asthma, may thus be a simplex normal. There is no known history of asthma in the mother's family. In the histories of the D., Cr., and Co. families (figures 9, 10 and 11, respectively), the asthma, from which

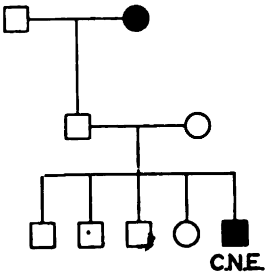

FIGURE 8

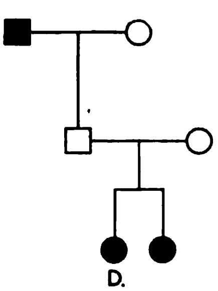

FIGURE 9

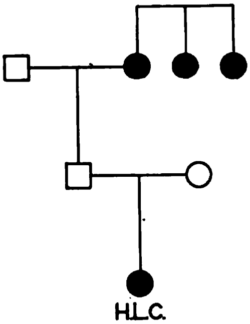

FIGURE 10

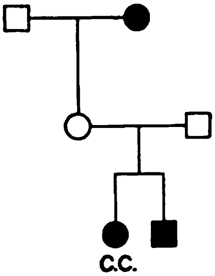

FIGURE 11

one of the grandparents suffered, skips the generation of the parents, and reappears in the patient. In the Ck. family, the parents are normal, the father's brother has asthma, and two of five children have asthma. According to the Mendelian law of inheritance, a dominant character appears in the direct line in every generation, but a recessive character shows itself in the body of the individual only when present in the germ cells in the duplex condition, inherited from both parents. In this group of family histories, bronchial asthma behaves as a recessive character, because it is transmitted to the offspring when both parents are normal, its origin being demonstrated in the family of one parent, and unknown in the other.

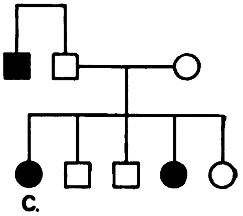

FIGURE 12

In the M. family (figure 13), asthma appears as a recessive character in the second and third generations, in that the offspring of apparently normal parents have asthma. But in the fourth generation the children of an asthmatic father and a normal mother have asthma, which might

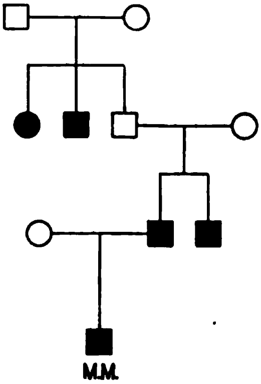

FIGURE 13

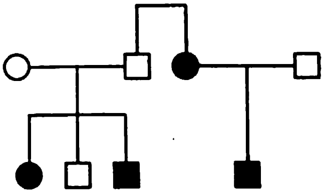

FIGURE 14

indicate that asthma is a dominant rather than a recessive character. In the Crz. family (figure 14), the asthmatic tendency behaves like a dominant character in that the son of an asthmatic mother has asthma, but like a recessive, where two of three children of non-asthmatic parents have asthma. In the P. family (figure 15), the father and paternal aunt of the patient had asthma, but the daughter of the normal brother and his normal wife shows beginning asthma with heavy colds. If the mother of the patient were a simplex normal, the patient might have inherited asthma from both father and mother, while her brother inheriting asthma only from the father is normal like the mother, but transmits the asthmatic tendency received from him. In the V. family (figure 16), the patient, R. V., a boy aged 14, is sensitive to pollen and lobster, has asthma in the summer; the parents are normal, but the father's sister has hay-fever and is sensitive to pollens. Thus the father in all probability is a simplex normal, transmitting the tendency, but its presence in the mother's family cannot be shown.

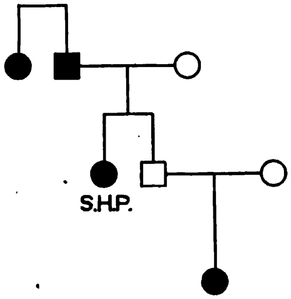

FIGURE 15

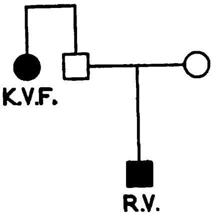

FIGURE 16

In the A. family, the oldest brother, a first-cousin, and the maternal grandmother have asthma. The oldest child of the asthmatic brother, whose wife is normal, has urticaria from eating egg, and the second child has eczema and asthma after eating egg, milk, and wheat. The patient, a girl, aged 17, has eczema, and had asthma as a child until four years of age, followed by a nearly free interval until she was eleven years old, and a return of the condition since that time. By cutaneous tests she is sensitive to cat hair and wheat, and she improved when removed from contact with cats and when wheat was omitted from her diet. The parents of the patient and her brother, and of the asthmatic first-cousin, are all non-asthmatic, the tendency may have been transmitted from the grandmother through the mother to the patient and her brother, and through the father of the cousin. Where both children of the asthmatic brother show protein sensitization, one as asthma and the other as urticaria, the simplest explanation is that the tendency is dominant over the normal condition; but it is not impossible that the mother transmits asthma also.

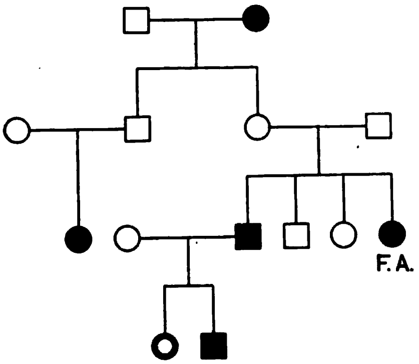

FIGURE 17

In the group of family histories presented next in order, the asthmatic tendency is found in the families of both parents. In the B. family, the maternal grandmother and her sister were asthmatic. She married a man of unknown family history, but who according to the theory that asthma is recessive, must have transmitted the asthmatic tendency, because their daughter, the mother of the patient, had asthma. The father of the patient was normal, but since his sister had asthma, he probably was a simplex normal and transmitted the tendency. The patient, a woman, aged 41, who has had asthma for nineteen years, is not sensitive to any proteins tried. Her husband is normal, and none of the four children show any tendency to asthma or protein sensitization. Thus the asthmatic tendency in the patient is probably recessive in her children to the normal condition inherited from her husband; but theoretically all of them bear the asthmatic tendency recessive in their germplasm and are capable of transmitting the tendency to half their offspring. This family history may be interpreted in a different fashion, as were other family histories earlier in the paper where asthma appeared for three generations in the daughters of asthmatic mothers and normal fathers. If it is assumed that the tendency to asthma is dominant over the normal condition, the asthma inherited from the maternal grandmother is dominant over the normal condition in the maternal grandfather, and hence their daughter, the mother of the patient is asthmatic. Under this theory, the father of the patient, although his sister had asthma, was normal. In the patient, the normal condition transmitted from the father is recessive to the asthmatic condition inherited from the mother. Marrying a normal man, she would be expected to transmit the asthmatic condition to half her offspring, and the other half, inheriting the recessive normal condition from both father and mother, would be normal and incapable of transmitting asthma. The difficulty in the way of this theory, that all the children are normal, is not insurmountable, because the children are still young, and may develop asthma later in life.

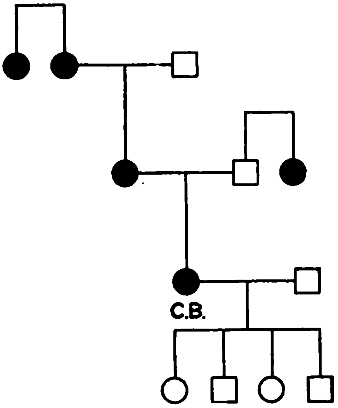

FIGURE 18

In the H. family, the paternal grandparents both had asthma; two sisters of the paternal grandfather, and the father of the paternal grandmother, were asthmatic; hence asthma was present in the families of both parents of the father, and this couple is more heavily tainted with the condition than any other pair found in the family histories. Of their five children, four had asthma. A non-asthmatic daughter, marrying a normal man had two children, both with asthma. The father of the patient, marrying a normal woman, who does not know of the occurrence of asthma in her family, has three children, of whom the oldest, the patient, a girl aged eight, has asthma, but is not sensitive to proteins. An asthmatic daughter, marrying a normal man, has seven children, six normal and one with asthma. Under the theory that asthma is a dominant trait, if both paternal grandparents were simplex dominants, the mating would be like type 4, and three-fourths of their children would be expected to have asthma, and one-fourth to be normal, which is exactly what happened; but the normal children should not be capable of transmitting asthma; but the normal daughter did transmit asthma to her two children. If the paternal grandfather were a duplex dominant, and the paternal grandmother a simplex dominant, as seems probable from the fact that her mother was normal, this mating would correspond to type 2; in that case all the children would be expected to have asthma, but one-half would be simplex; in this case the non-asthmatic daughter is not provided for. If asthma is recessive to the normal condition, the paternal grandparents must have both been pure recessives, and in that case the mating would correspond to type 6; the normal mother of the maternal grandmother would have had to be simplex, transmitting asthma, by this hypothesis, and the non-asthmatic daughter is not provided for. By the recessive theory also, all the normals who were married by these offspring would have to be simplex, in order to account for the appearance of asthmatics in each of the three families of grandchildren. But the theory that asthma is a dominant character does not provide for the two children of normal parents in the first family, and in the third family

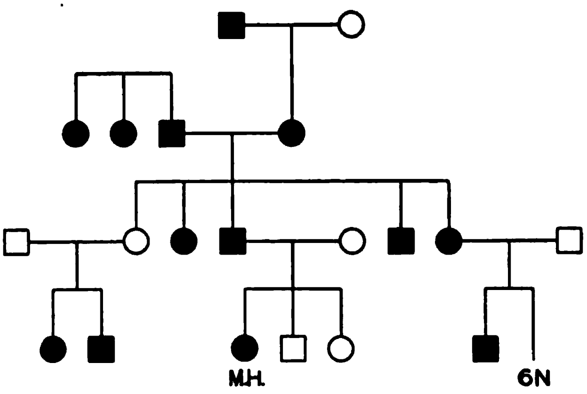

FIGURE 19

half of the offspring should have been asthmatic, instead of one out of seven. All the grandchildren are young and may develop the trait later. On the whole, the theory that asthma is recessive seems to explain more of the facts in this remarkable family history, than the opposite theory.

In Moon, the patient is the daughter of an asthmatic mother and a normal father, the father's father had asthma, and hence he may have transmitted the tendency. All the children of the normal paternal grandmother and the asthmatic paternal grandfather were normal, so in that mating at least, the asthma was recessive to the normal condition. If the father of the patient were a simplex normal, able to transmit asthma to half his offspring, and the mother whose parents and four brothers were normal, was a duplex recessive, transmitting asthma to all her offspring, their mating corresponds to type 5, where half the children would be expected to be pure recessive, that is asthmatic, and half, while normal, would transmit asthma. The facts of one asthmatic out of three correspond fairly closely. The normal condition of the husband of the patient is apparently transmitted to their normal grown children. This family history could of course be interpreted by the opposite theory.

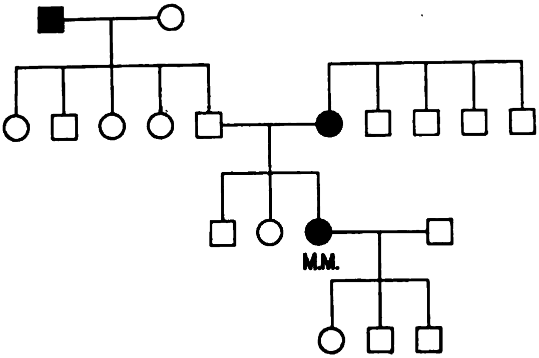

FIGURE 20

In Biggs, the patient, G. B., a girl aged 18 has suffered from asthma and eczema all her life, and she is sensitive to horse hair, various foods, bacteria and pollens. Her brother, R. B., aged 21 has hay-fever, sometimes with asthma, and he gives positive reactions with pollens. The mother, and her brother and mother had asthma. The father has no asthma, hay-fever, or other sensitization to pollens, but his brother has asthma and his sister has hay-fever and gives positive cutaneous reactions to the pollens. By the theory that asthma is recessive to the normal condition, the mother of the patient would be supposed to be a pure recessive, inheriting asthma from her asthmatic mother and normal father; the father of the patient would be expected to be a simplex normal capable of transmitting the asthmatic tendency present in his family. If this is true, the older son who is normal, inherits the dominant normal condition from his father, but carries the asthmatic tendency inherited from his mother. His asthmatic and sensitive brother and sister inherit the tendency from both father and mother. This seems the most reasonable interpretation of the family history. If the normal condition is recessive to the asthmatic condition, the father cannot transmit the character, and the fact that it occurs on his side of the family has no bearing on the case.

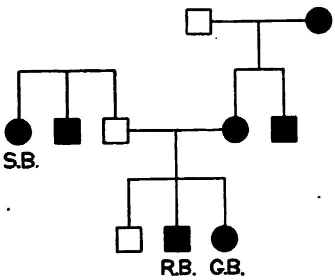

FIGURE 21

In Johnstone, the maternal grandfather was asthmatic, and he had two asthmatic brothers; hence by our theory, he was a duplex recessive. Marrying a normal woman, whether a simplex or duplex normal cannot be determined from the history, he had a normal daughter, who by the theory was a simplex normal, capable of transmitting the asthmatic tendency. Her husband was normal, but probably simplex, because his sister had asthma. Of the four children of this pair of normal simplex individuals, a daughter, the patient, has hay-fever from, and by the cutaneous tests is sensitive to, cat hair; a brother has asthma, a sister has eczema, and a second son is normal, without evidence of protein sensitization. One of the asthmatic uncles of the mother married a normal woman, but his son has summer hay-fever and his daughter has cat hay-fever. It is impossible to explain this family history on the hypothesis that asthma is dominant to the normal condition, for in that case neither

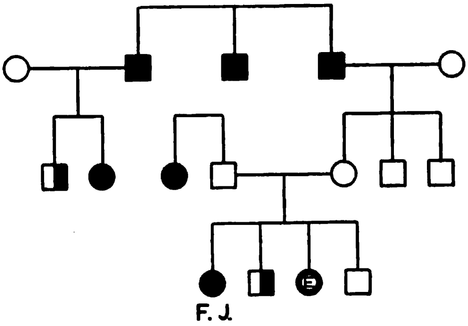

FIGURE 22

parent would be capable of transmitting the asthma which is shown to be present in their families.

In Tucker, the asthmatic father married an apparently normal mother, who probably was simplex, inheriting asthma from her father. Of the three children of this couple, two have asthma, inherited, if asthma is recessive, from both father and mother. The normal child probably has inherited the asthmatic tendency from the father, but not from the mother. The asthmatic brother and sister are both sensitive to proteins.

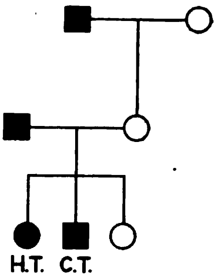

FIGURE 23

In the Guppy family, the maternal grandmother of the patient had asthma, but her husband and all their eleven children were normal, the asthmatic tendency in this mating behaving as a recessive character. Probably at least some of the children were capable of transmitting the tendency; theoretically all of them would have been simplex, if asthma is recessive; if it is dominant, at least half of them should have been asthmatic. The mother of the patient, normal but probably capable of transmitting asthma, married an asthmatic, the son of normal parents, who according to the theory that asthma is a recessive character must have inherited the tendency from both parents, simplex normals bearing the tendency recessive in their germ-plasm. If the father was a duplex asthmatic, and the mother of the patient a simplex normal, the theoretical expectation would be that half the children would be asthmatic, and the other half normal but bearing the asthmatic character in their germ-

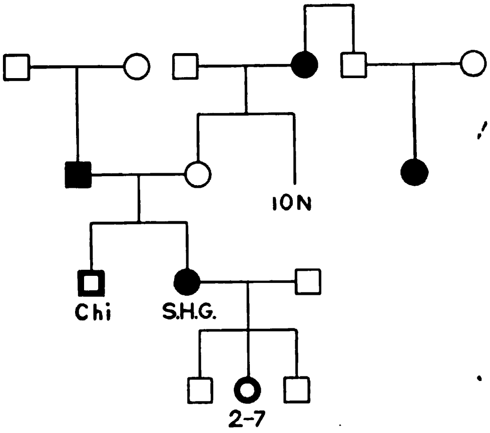

FIGURE 24

plasm. The two children of the mating were both asthmatic. The patient's brother had asthma in childhood, but recovered as he grew older. The patient, S. H. G., did not develop asthma until she was 26, but she has had asthma now for sixteen years, and by the cutaneous tests she is sensitive to horse, cat and bacteria. Thus both the patient and her brother show the asthmatic tendency, inherited perhaps from both parents. Her husband is normal and there is no history of asthma in his family. If he is a duplex normal and she a duplex asthmatic, all the children would be normal, but simplex and capable of transmitting asthma. If he is a simplex normal, he would transmit asthma to half his children, which, uniting with the tendency transmitted by the mother would mean half the offspring asthmatic, and half simplex normal. Of their three children, one, the daughter, had asthma with colds from the age of three to seven, but she has recovered as she grew older. In this family history, therefore, the asthmatic tendency behaves according to the Mendelian law, as an inherited trait recessive to the normal condition.

In Foye, both paternal grandparents had hay-fever; their son, inheriting the tendency perhaps from both parents, was asthmatic. The patient, the daughter of this asthmatic father and a normal mother has asthma, but is not sensitive to proteins. The first-cousin of the father, whose father had hay-fever, has hay-fever also. So in this family history the asthmatic tendency seems to behave consistently as a dominant character and the recessive theory can be made to apply only by supposing that the normal mother of the patient, and the normal father of the cousin with hay-fever, were both simplex normals and transmitted the tendency to their offspring.

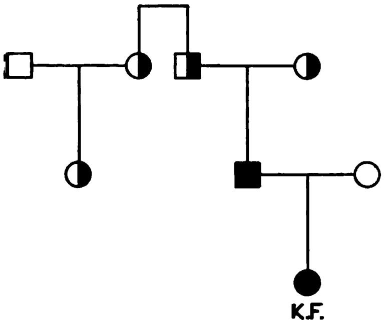

FIGURE 25

In the B. family, the maternal grandfather of the patient had asthma, his wife was normal, and their daughter, the mother of the patient had asthma, which would be expected if asthma is dominant; but which can be explained, if asthma is recessive, only by supposing that the maternal grandmother was simplex and able to transmit asthma. The father of the patient, the son of two normal individuals, has asthma, a condition which cannot be explained by the theory that asthma is dominant. On the theory that asthma is recessive, the appearance of asthma in the children of normal parents can be explained. Both parents are heterozygous, and their mating corresponds to type 4; three-fourths of their offspring would be normal, but two-thirds of these normal offspring, or one-half the entire number, would be simplex normals, able to transmit asthma, the other normals would be duplex, not carrying asthma in their germ-plasm; one-fourth the offspring would be asthmatic, that is pure recessives, and would transmit the tendency to all their offspring. The father of the patient falls in this latter group. Both children of these asthmatic parents show the asthmatic tendency; the patient, a boy, aged eight, has had bronchitis all his life and asthma for the past two years, and his younger brother has bronchitis and is beginning to choke up with it. There is no evidence of protein sensitization in this family. Whether asthma is dominant or recessive cannot be judged from this last mating, because if asthma is dominant, the children of asthmatic parents would be expected to show the trait in all cases, if the germ-plasm were pure, and in all but one-fourth of the offspring, even if both parents were simplex, while if the asthmatic condition is recessive, the asthmatic patients must be pure recessive, and not capable of transmitting the normal condition.

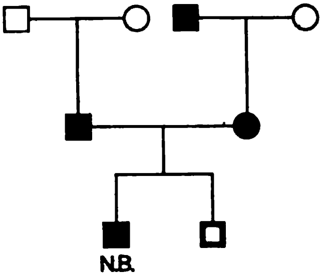

FIGURE 26

In Chadwick, the family history looks again like inheritance of a dominant trait. The patient, M. C., and her brother, both suffering from asthma, were the children of an asthmatic father and a normal mother; of her eight pregnancies, three children are normal, a daughter of four is choked up at times, one child died in infancy, and there were three miscarriages. Thus in the first generation, the asthma behaves like a dominant character, while in the third generation it is recessive to the normal condition in three out of four children. If asthma is a recessive character, for it to appear in the direct line in every generation, as it does in this family, the normal mother of the patient and her normal husband must have been heterozygous, carrying asthma in the simplex condition in their germ-plasm, and transmitting it to half their offspring.

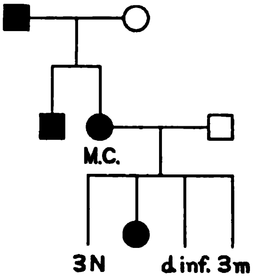

FIGURE 27

In Shewmann, an apparently normal maternal great-grandfather married an asthmatic, and of their ten children, one had asthma, and he had only wheezing and shortness of breath with colds. One apparently normal son, marrying a normal wife, had a son who suffered from asthma. A normal daughter, marrying a normal, had five children, of whom two are normal, one daughter, the patient, M. L. S., has asthma and eczema and is sensitive to bacteria and some foods; a son has hay-fever, and in his youth had asthma, with colds; another daughter, non-asthmatic, and non-sensitive, has frequent colds. The patient, whose husband was normal, has three children, none of whom as yet has developed asthma, or any evidence of protein sensitization. In this family history, the asthmatic tendency does not behave like a dominant character; in the mating of the grandparents, if asthma were dominant and the grandmother were duplex, all the children should have had asthma, the mating then corresponding to type 3; if the grandmother was simplex, the mating would correspond to type 5, and at least half the offspring would have been expected to have asthma. If asthma is dominant, the two instances of asthmatic children born of normal parents cannot be explained, and at least half the children of the patient should develop asthma. If asthma is a recessive character, the normal grandfather, the mother of the asthmatic first-cousin, and the father of the patient, must all be supposed to have been simplex normals, capable of transmitting asthma; that the father of the first-cousin and the mother of the patient were simplex normals is rendered probable by the occurrence of asthma in their families.

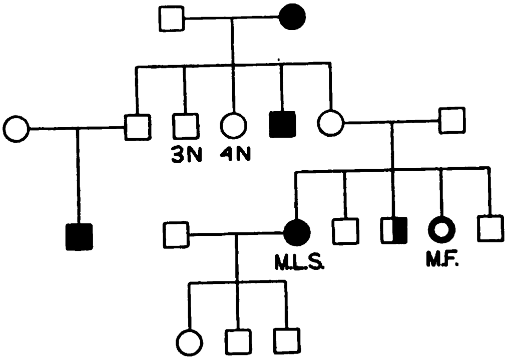

FIGURE 28

In the family histories which follow, the data concerning normal members of families is incomplete, and for that reason proportions and percentages of offspring in given matings cannot be compiled for all cases. For this defect they are presented separately, although they are not different in any way from the three groups which have preceded. In the C. family, the father had slight asthma and three sons have asthma, while the son of the mother by a former marriage, and a son and daughter by this second marriage have no asthma. In the mother's family, there is no known history of asthma. Here the asthmatic condition seems to be dominant, in that three out of five children apparently inherit it from the father, while the two normal children inherit the recessive condition from both parents. If the asthmatic condition is to be interpreted as a recessive trait in this family, it must be supposed that the mother was simplex and transmitted asthma to half her offspring, which seems a far-fetched theory. The patient, V. E. C., a man aged 31, has had asthma for the past seven years, with a history of sudden onset after an operation for appendicitis; he is sensitive to pollens and bacteria, and gives a slightly positive reaction to horse hair. The wife and four young children of this patient are normal. His brother, C. W. C., aged 25, has had asthma for fifteen years and is sensitive to horse hair and to red-top pollen.

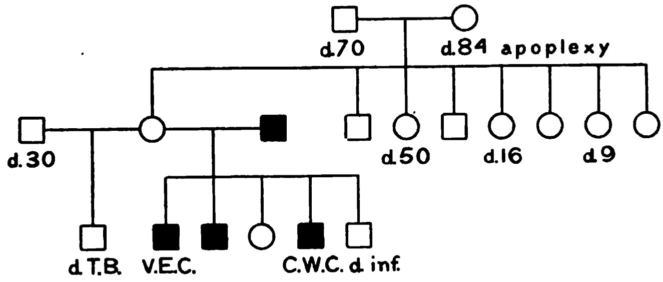

FIGURE 29

In the Newell family, the mother had hay-fever but has gradually recovered; the patient, M. N., a woman aged 46, has fall hay-fever and with it asthma and sneezing and running of the eyes and nose when near horses; in childhood she had eczema. By the cutaneous tests she is sensitive to horse and pollens. A brother has asthma all the year; another brother, J. A. N., aged 36, has both spring and fall hay-fever, and is sensitive to pollens. Here the tendency to hay-fever inherited from the mother shows itself in three of her five offspring, one daughter having asthma and hay-fever, one son asthma, and another son hay-fever, while two daughters are normal. In this family the asthmatic tendency appears to act as a simple Mendelian dominant, the two normal daughters inheriting the recessive normal condition from both parents. If it is to be interpreted as a recessive character, the

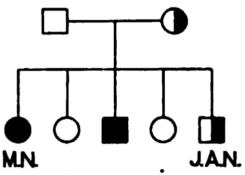

FIGURE 30

father, although normal, must be supposed to have been simplex, transmitting asthma to three of his five children.

In the Smith family, the father had hay-fever, the mother had asthma, the son hay-fever, and the daughter asthma. There may have been normal children not noted in the history, but taken as it stands the history looks like the inheritance of a pure trait, where both parents are duplex, transmitting the character to all their offspring, and by this interpretation, the mating might correspond to either type 1 or 6, the asthma and hay-fever being either dominant or recessive in this family.

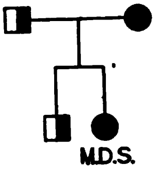

FIGURE 31

In the Poe family, the mother and maternal grandmother of the patient had hay-fever and the maternal great-grandfather had asthma. In the father's family, an uncle of the patient had hay-fever. The patient, a girl aged 24, has had asthma for nineteen years, and is sensitive by the cutaneous tests to horse hair and pollens. Her brother and his three children are normal. If asthma is a dominant trait in this family, it can be shown to have been passed down the direct line for four generations from parent to child, in each generation prevailing over the normal condition inherited from the other parent. In the last family, the daughter has inherited the asthmatic tendency from the mother and the normal from the father, while her brother has inherited the recessive normal tendency from both parents, and is not capable of transmitting it to his offspring. If asthma is dominant over the normal condition, the fact that the father's brother had hay-fever has no bearing on the case. But if asthma is a recessive character, the normal father may have been simplex, transmitting the normal condition to his son and the asthmatic tendency to the daughter. By this latter hypothesis, the mother would have to be duplex, transmitting the asthmatic character to all her children. The daughter, inheriting the tendency from both parents, is asthmatic; the son, normal like his father, bears the asthmatic character recessive in his germ-plasm and theoretically transmits it to half his offspring, but if his wife is a pure or duplex normal, none of the offspring will show the tendency. The hypothesis that asthma is recessive in this

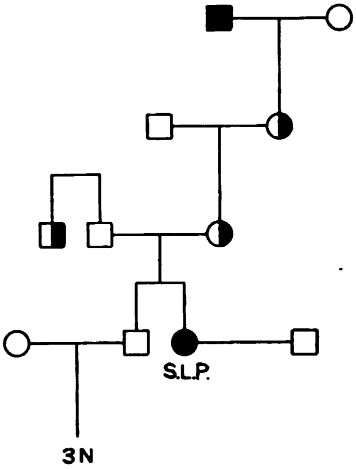

FIGURE 32

family makes necessary the supposition that the maternal great-grandmother and the maternal grandfather, in addition to the father of the patient, must have been simplex.

In the Brown family, the maternal grandfather had asthma, and four of his seven daughters were asthmatic. Three daughters, including the mother of the patient, and all six sons were normal. Although both parents were normal, the patient, A. B., a man aged 40, has had asthma for two years, and is sensitive to bacteria. In the generation of the parents, the asthmatic character behaves as a dominant trait in four of the thirteen children. But if asthma is dominant, the fact that the maternal aunts and grandfathers had asthma, would not explain its appearance in the grandson, skipping the mother. On the other hand, if asthma is recessive, the maternal grandfather must have been a duplex asthmatic and his wife a simplex normal, capable of transmitting asthma; then four of the thirteen children would have inherited asthma from both parents, while nine would have inherited asthma from the father and the normal condition from the mother, and would be expected to transmit asthma to half of their children. In this case also, the father of the patient would have had to be a simplex normal.

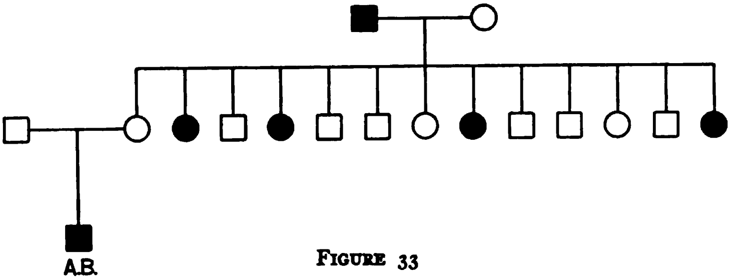

FIGURE 33

In the following family histories, the parents of the patients are normal, but the asthmatic character appears collaterally in the family of one parent. In the H. family (figure 34), the patient, a woman, aged 37, has had asthma for two years, and is not sensitive to proteins; of her maternal

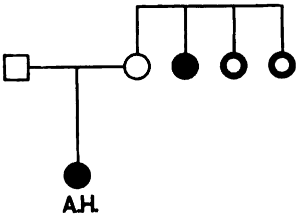

FIGURE 34

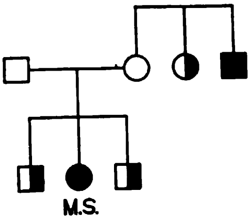

FIGURE 35

aunts, one has "wheezing spells like asthma," and two are made ill by eating eggs, In the S. family (figure 35), the patient, a woman, aged 25, has had asthma since she was three years old, and she is sensitive to horse hair, pollens, potato, and cereals. The two brothers of the patient have hay-fever, her maternal uncle has asthma, and her maternal aunt has hay-fever. In the G. family (figure 36), the patient, a boy, aged 12, began to have asthma at two years of age, and as an infant he had eczema. By the cutaneous tests he is sensitive to animals, pollens, wheat, and egg. One brother has fall hay-fever, another who had eczema in infancy has sneezing and running of the nose and eyes and a little choking up when he is near horses. The father's sister has hay-fever.

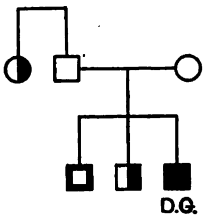

Figure 36

In the R. family, the asthma appears to have skipped two generations because both the father and the father's father were normal, but the brother of the latter had hay-fever. The patient, a man aged 40, has had fall hay-fever with asthma for sixteen years, and is sensitive to pollens; his sister has spring hay-fever, and a normal sister has running of the nose. The asthmatic character cannot be interpreted as a dominant trait in this last or the preceding family histories. In this last family, if the grandfather were a simplex normal, as appears possible from the presence of hay-fever in his family, he would have been expected to transmit the trait to half his offspring, but if his wife were a duplex normal, none of the children would have been asthmatic. If the father of the patient inherited the normal tendency from his mother and the asthmatic tendency from his father, he would have been a simplex normal and capable of transmitting asthma to half his offspring; and if his wife also were a simplex normal, the patient and his sister with hay-fever would have inherited the tendency from both parents, while the normal sister inherited the normal condition from one and perhaps from both. The wife and small son of the patient are normal.

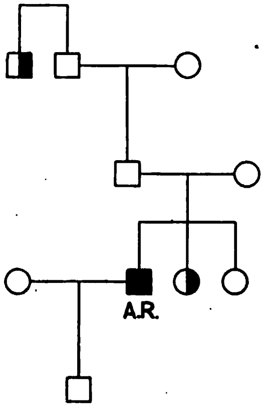

Figure 37

In the A. family, the history is exactly the same as in the preceding family, except that the asthmatic character skips two generations in the mother's family instead of the father's, the sister of the maternal grandmother having asthma, and in the fact that a first-cousin on the father's side has asthma; this makes more plausible the hypothesis that the father is a simplex normal capable of transmitting asthma to half his offspring. In the last four family histories and in part of the P. and the S. families, the asthmatic tendency has behaved as a recessive Mendelian character.

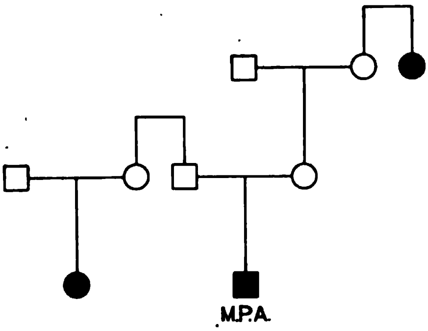

FIGURE 38

Thirty-eight family histories are presented with a more or less complete record of ninety-one matings in which parents or offspring are asthmatic or have hay-fever. In three matings, one producing only one child, both parents and all the children were asthmatic, five children in all being produced by this type of mating. In one mating, where both parents had asthma, four children were asthmatic and one was normal. In no mating where both parents were asthmatic were all the children normal. In nineteen matings where one parent was asthmatic, all the children were asthmatic, but in twelve cases only one child was born; from this group of matings twenty-five asthmatic children were produced. In nineteen families where one parent was asthmatic were born thirty-five asthmatic and sixty-seven normal children. In seventeen families where one parent was asthmatic, which included six matings where only one child was born, all the sixty children produced were normal. In twenty matings where both parents were normal, all the children were asthmatic, but this included thirteen matings with only one child; from this group, were produced in all thirty-one asthmatic children. In twelve matings where both parents were normal, fifteen normal children were born and thirteen asthmatic children.

Where both parents had asthma or hay-fever, out of four matings in which ten children were born, only one child was non-asthmatic, and that child, although she married a normal, produced two children, both asthmatic. Hence the evidence seems strong that where both parents show the asthmatic condition, the character tends to breed true; if the asthmatic character is recessive to the normal condition, these four matings must be of type 6, and it is difficult to account for the one normal child by this hypothesis. If asthma is a dominant character, the matings which produced only asthmatic children might have been either type 1 or type 2, while the mating in which the normal daughter was born was probably type 4, where the theoretical expectation would have been three asthmatic children to one normal; the actual ratio of one normal to four asthmatic is very close. In nineteen matings where one parent was normal and one asthmatic, twenty-five asthmatic and no normal children were produced; if asthma is a dominant character, this group would correspond exactly to mating of type 3, but if asthma is a recessive character this group is more like type 5, and in that case half the children would have been expected to be normal; the fact that in twelve of the seventeen matings there was only one child and in the remaining six only two each, makes this latter hypothesis somewhat more tenable. In nineteen matings, where one parent was normal and one asthmatic, sixty-seven normal and thirty-five asthmatic children were produced, a ratio of a little less than two normal to one asthmatic. If asthma is a dominant character, this type of mating would correspond most nearly with type 5, in which the theoretical expectation would be half the offspring asthmatic and half normal; the preponderance of normal offspring might be partly discounted by the fact that some of them are still very young and may develop the asthmatic condition later in life. If asthma is recessive to the normal condition, this group would still correspond most closely to type 5. In seventeen matings where one parent was normal and one asthmatic, sixty children were produced, all normal, an impressive number when compared with the mating of the same type where seventeen similar pairs of parents produced twenty-two asthmatic offspring. In this group, if asthma is a dominant character, the matings are necessarily either of type 2 or type 5, and the results correspond more nearly to type 5, where only half of the offspring would be expected theoretically to show asthma; here again some children are young and may develop the disease later on. But if asthma is recessive to the normal condition, this group would correspond exactly with type 3, where the theoretical expectation would be all normal.

The two groups of matings where both parents are normal, but have asthmatic children, cannot be explained by the theory that asthma is dominant to the normal condition. Twenty-two pairs of normal parents produced thirty-one asthmatic children, distributed in twelve families of one child, five of two and three of three children each. This group comes nearest to the mating of type 4, but even then it would have to be supposed that only the recessive fourth of the expected offspring had been born, while three times as many normal children as asthmatic would be the theoretical expectation. The twelve pairs of normal parents who bore fifteen normal and thirteen asthmatic children corresponds most closely to the type 4 mating; out of twenty-eight offspring the theoretical expectation would have been twenty-one normal to seven asthmatic children.

A family history published by DRINKWATER (1909) not different in any essential from the family histories of asthma presented in this paper, shows asthma transmitted in the direct line for three generations, and in three families born of one normal and one asthmatic parent, exactly half the offspring have asthma and half are normal. while two other such pairs have normal children. He shows no asthmatic children born of normal parents. Three of his matings correspond to type 5, and two are of type 3, if asthma is taken to be recessive to the normal condition.

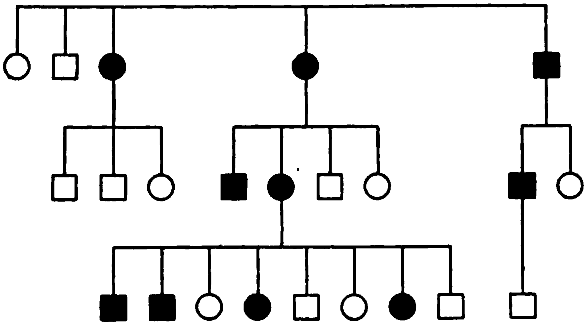

FIGURE 39

In the thirty-eight family histories presented above, the asthmatic condition behaves as a Mendelian dominant in seven; in twenty-five the character must be interpreted as a recessive character, if it is to be considered as inherited at all, while in six families it may be interpreted either way. In the more complete histories, both types are shown to occur in the same family, sometimes it behaves as a dominant, appearing in the direct line in two or more generations, and again as a recessive in that it skips one or more generations, or is present collaterally but not in the direct line. In the families most heavily tainted on both sides, the proportion of asthmatic offspring is greater than in families where it is present on only one side, or collaterally. By assuming that some normal individuals are simplex and transmit the character without themselves showing it, most family histories in which asthma occurs can be interpreted in accordance with the Mendelian law of heredity; the asthmatic trait behaves as a recessive character. But when an attempt is made to group the family histories according to the theoretical matings, the facts do not always meet the theoretical expectation. Where both parents have hay-fever or asthma, the correspondence with type 6 is very close, although two normal children are shown, born of matings where both parents have hay-fever or asthma. Where one parent is normal and the other asthmatic, 127 normal children were born to sixty asthmatic, so that the normal condition prevails over the asthmatic in a ratio of more than two to one. The group where a normal and an asthmatic parent have all normal offspring would correspond exactly to type 3 if asthma were dominant, but since asthma behaves as a recessive, this must be taken as a variety of type 5 mating, where the expected normal half of the family was not born, and this view gains support from the small number of children and the fact that most of them are only children. If this group is united with the group where one asthmatic and one normal parent bear some children normal and some asthmatic, the typical type 5 condition, there are 60 asthmatic to 67 normal children, almost the theoretical expectation of one dominant to one recessive. Where from a normal and an asthmatic parent all the children are normal, the condition corresponds exactly to the theoretical expectation in type 5. Where both parents are normal but have some or all children asthmatic, the group corresponds most closely to the type 4 mating, with forty-five asthmatic offspring to fifteen normal, or three recessive to one dominant, which is the reverse of the expected condition. To summarize then, interpreting asthma as a recessive to the normal condition, seventeen matings of type 3 are shown, with 60 normal offspring; thirty-four matings of type 4, with fifteen normal and forty-five asthmatic offspring; thirty-four matings of type 5, with sixty asthmatic and sixty-seven normal offspring; and four matings of type 6, with nine asthmatic and one normal child, who transmitted asthma. No family history is presented which cannot be interpreted by the theory that asthma is recessive. By this theory also the apparently sporadic cases of asthma which occur frequently in families where no other case is known can be explained that the parents in question happen to be not pure but simplex normals, the recessive trait which each bears being transmitted from both to the child who develops asthma, but from only one to all the other children, a theoretical chance of one in four. Thus the 52 percent of cases with a negative family history can be brought under the same law of inheritance as those with a known history of asthma in the family.

SUMMARY

In a series of 400 cases of bronchial asthma, 192, or 48 percent, gave a positive family history; in the group of 191 patients sensitive to proteins 52 percent gave a positive family history while among the 209 non-sensitive cases only 41 percent gave a family history of asthma; hence asthma of the anaphylactic type has a greater tendency to run in families than does the non-sensitive asthma. Asthma is inherited with equal frequency from father and mother; from the father's family 66 patients may have inherited asthma, 39 directly, 8 skipping a generation, 1 skipping two generations, and 18 collaterally. From the mother's family, 64 patients may have inherited asthma, 25 directly, 22 skipping a generation, and 17 collaterally. A nearly equal number of men and women inherit asthma from the father, but twice as many women as men inherit asthma from the mother, and 25 percent more women than men inherit asthma directly from the parents.

In both sensitive and non-sensitive groups a large number of patients who began asthma before thirty, are found to have a positive family history, and a larger proportion with an age of onset after thirty are found among those with a negative family history. An earlier age of onset is found among cases with a positive family history, and children of asthmatic parents who develop the disease begin asthma earlier than did their parents. There is no distinction in kind, variety or degree of sensitization to foreign proteins as demonstrated by the skin test, between sensitive patients who give a family history and those who do not. From the presence or absence of asthma in the family history, it is not possible to draw conclusions as to the cause, severity of the disease, or prognosis in a given case of bronchial asthma.

Sensitive and non-sensitive asthma do not run pure in the family histories, but both types occur in the same family; hence, from the standpoint of heredity, these two types of asthma and other manifestations of sensitiveness to proteins, such as hay-fever, and some skin disturbances, must be accepted as equivalent.

Twenty-two cases are presented where more than one member of a family has been tested on the skin with proteins; 14 pairs of relatives are both sensitive to some protein; in three pairs, both are non-sensitive; in five pairs, one is sensitive, the other not. Of the 14 sensitive pairs, 6 do not react to the same proteins, and the varying reactions correspond to the different clinical symptoms in the individual. In eight pairs who react to the same protein, in five cases the specific protein is a pollen and the identical skin tests are explained by the hay-fever and pollen asthma from which these patients suffer; in the other three pairs giving identical skin tests, all individuals show multiple sensitization, and the test is identical with respect to only one protein. Sensitization to specific protein is not transmitted from parent to child, or from pregnant mother to offspring. Parent may be sensitive and child non-sensitive, or *vice versa*. The sensitization is not transmitted even when the asthma seems to be inherited. The skin test is an index of the cause and symptoms of the individual, but bears no specific relation to the family history or to the problem of inheritance. Hence histories of hay-fever patients would seem to give evidence of the inheritance of protein sensitization, when really the identical symptoms explain the identical test, which, since there are only two principal causes, the pollens of ragweed and of timothy, must be identical in at least half the cases and will be identical in an even larger proportion of cases, since ragweed is by far the more common cause.

In the thirty-eight family histories presented, recording ninety-one different matings, the asthmatic tendency behaves as a recessive Mendelian character. Where both parents have asthma or hay-fever, all the children tend to show the condition. Where one parent has asthma and the other is normal in seventeen matings all the children were normal, 60 children in all; in a second group where one parent has asthma and the other is normal but probably simplex, half the offspring are normal and half are asthmatic, really 60 asthmatic to 67 normals. Where both parents are normal, but can be shown or must be assumed to be simplex, there were three times as many asthmatic as normal children, the reverse of the theoretical expectation but explained in part by incomplete records of normal individuals. By the theory that the tendency to asthma is recessive to the normal condition, even the sporadic cases which arise in normal families can be explained on the assumption that the two parents are simplex, and the asthmatic child inherits the character from both parents, the typical recessive expected in one-fourth the offspring of heterozygous parents.

To the practitioner therefore, the family history lends no aid on the individual case of bronchial asthma. With closer methods of diagnosis, the line between true bronchial asthma of the sensitive type and the non-sensitive asthma due to bacteria will be more tightly drawn; the family history will be more accurate, and the number of positive family histories will diminish. Thus eventually only the expected one-fourth of the offspring of normal parents who are capable of transmitting the disease will be reported. From the standpoint of eugenics, the advice against the marriage of two people who have a similar defect, particularly if they are related, holds good; but since over 50 percent of the cases gave a negative family history, this precaution would not prevent a large number of cases.

The nature of the inherited factor is unknown, whether it is the presence of something not found in the normal individual, or the absence of something usually present, all the theories of antibodies and protein split products fail to explain. Since sensitization to proteins is not inherited, it is a tendency or a power to develop asthma which is inherited.

CONCLUSIONS

1. In a series of 400 cases of bronchial asthma, 48 percent gave a history of asthma in the family and 52 percent gave a negative family history.
2. Asthmatics of the sensitive type more frequently give a positive family history than do non-sensitive cases.
3. Asthma is inherited with equal frequency from the family of either parent. An equal number of men and women inherit asthma from the father, but in this series, twice as many women as men inherit asthma from the mother, and 25 percent more women inherit asthma directly from the parents, without skipping a generation.
4. An earlier age of onset is found among patients with a positive family history.
5. Children of asthmatic parents that develop the condition begin asthma earlier than did their parents.
6. There is no distinction in kind, variety, or degree of sensitization to foreign proteins as demonstrated by cutaneous tests, between sensitive patients with a positive family history and those with a negative history.
7. From the family history it is not possible to draw conclusions as to the cause, severity of the disease, prognosis or treatment in any given case of bronchial asthma.
8. Sensitive and non-sensitive asthma run pure in the family histories only occasionally, but commonly both types occur in the same family without distinction or rule, hence, from the standpoint of heredity, these two types of asthma, and also hay-fever and some urticarias and eczemas caused by the ingestion of protein, must be accepted as equivalent.
9. According to cutaneous tests with proteins, members of a family may be both sensitive, or both non-sensitive, or one sensitive and the other non-sensitive.
10. Even when both members tested are sensitive, they are only rarely sensitive to the same protein.
11. Sensitization to specific proteins is not transmitted from parent to child.

12. Sensitization to foreign protein may run in families, but just as frequently it is found in only one member of a family.

13. Even when protein sensitization does run in a family, it is not identical as regards either specific proteins or the clinical symptoms developed among different members of the family.

14. The cutaneous test is an index of the cause and symptoms of protein sensitization in the individual, but bears no relation to the family history or the problem of inheritance. In family histories of hay-fever, different members of a family give identical cutaneous tests because they have the same symptoms; moreover, since there are only two common causes of hay-fever—the pollens of ragweed and timothy—tests and symptoms must be identical in at least 50 percent of the cases, and they will be identical in an even larger proportion of cases because ragweed hay-fever is by far the more common condition.

15. In the family histories presented, where the diagnosis of asthma except in the patients examined in this clinic, is based entirely on the clinical symptoms, the asthmatic condition is found not to be congenital or transmitted by the mother to the foetus or through the milk, but it behaves as a true inherited trait, transmitted in the germ-plasm of both parents alike, and following closely in the family histories the theoretical expectation of a Mendelian character recessive to the normal condition.

16. Where both parents have asthma or hay-fever, all the children tend to develop the condition.

17. Where one parent is asthmatic, and the other parent, although normal himself, has asthma in his family, and hence carries the asthmatic tendency recessive in his germ-plasm, half the children are asthmatic and half are normal but simplex, capable of transmitting the asthmatic tendency to half their offspring.

18. Where one parent is asthmatic and the other is normal and has no asthma in his family, all the children are normal but simplex.

19. Where both parents are normal but have asthma in their families and hence are probably simplex, theoretically one-fourth the children born would be asthmatic, and three-fourths normal; actually in this series, from this type of mating were produced three times as many asthmatic as normal children.

20. Where both parents are normal, but one is simplex, all the children would be normal, but half the children would be simplex and bear the asthmatic character recessive in their germ-plasm. Where both parents are duplex normal, all the children would be pure normal.

21. By the theory that bronchial asthma is inherited as a Mendelian trait recessive to the normal condition, the 52 percent of cases in this series, as well as the 51.6 percent in COOKE's series of 621 cases of sensitization, with a negative family history, can be interpreted. The asthmatic tendency can be transmitted if it is recessive, by individuals who are themselves not asthmatic, and it becomes manifest only in individuals who are duplex with respect to the character, that is, who inherit the tendency from both parents. Hence the tendency might be transmitted by several generations of normal individuals, but there is a theoretical expectation that when two normal persons, simplex with regard to the asthmatic tendency, marry, one-fourth their offspring will be asthmatic.

22. The nature of the inherited factor is unknown, whether it is due to the presence in the germ cells of affected persons of something not found in normal individuals, or the absence of something normally present, all the theories as to anti-bodies and protein split products have failed to explain. But it is the tendency or power to develop asthma, whether caused by sensitization to proteins or not, which is transmitted and not the condition itself.

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A DWARF MUTATION IN PORTULACA SHOWING VEGETATIVE REVERSIONS[1]

A. F. BLAKESLEE

Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York

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DWARF PLANTS

In 1914 Mr. B. T. Avery, who was then my gardener assistant at the Connecticut Agricultural College, called my attention to a single dwarf individual in a bed of red-flowered plants from commercial seed of a variety of *Portulaca grandiflora*. This plant formed a low compact pin-cushion-like growth in striking contrast to the long straggling habit of the normal plants near it. It is the origin of a series of dwarfs which we now have in a variety of colors, both singles and doubles. Commercial florists are on the lookout for dwarf plants for bedding purposes and such a cushion form as ours would likely have been noticed and propagated commercially by trained florists. So far as we have been able to ascertain, however, no drawf form of Portulaca is known to the trade. It is not unlikely, therefore, that the plant discovered by us was the first of its kind to be observed in this species.

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[1] A preliminary report on the present paper may be found under Blakeslee, A. F., and Avery, B. T., Jr., A vegetative reversion in Portulaca. Brooklyn Bot. Garden Mem. 1: 18. 1918.

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Description of dwarfs

Figure 1, at the right, shows two typical young dwarfs in 3-inch pots, in contrast to a normal plant of the same age at the extreme left. Dwarfing is brought about by an inhibition to the normal elongation of the internodes. Branching does not seem to be interfered with, and older plants may form dense mats 4 inches and more in diameter.

In normal plants, red in the stem is correlated with red and certain other colors in the flower. Typical dwarf plants in the adult stage, however, always have green stems. In young seedlings, both normals and dwarfs have similar primary stems, of a red color if destined to produce flowers correlated with red stems. Dwarfs beyond the seedling stage have no red in their stems. It was at first thought that their lack of color might be caused by the dense covering of leaves which kept out sufficient light to stimulate the production of the red color. Lack of illumination is not the only factor involved however, since stripping a dwarf stem of its leaves and removing them from the branch as soon as formed does not elicit red stem color in dwarfs even in direct sunlight. Moreover, reverting branches, to be discussed later, have red stems and often show a sharp line of color where they arise from a dwarf branch. It appears therefore that there is something peculiar in the make-up of

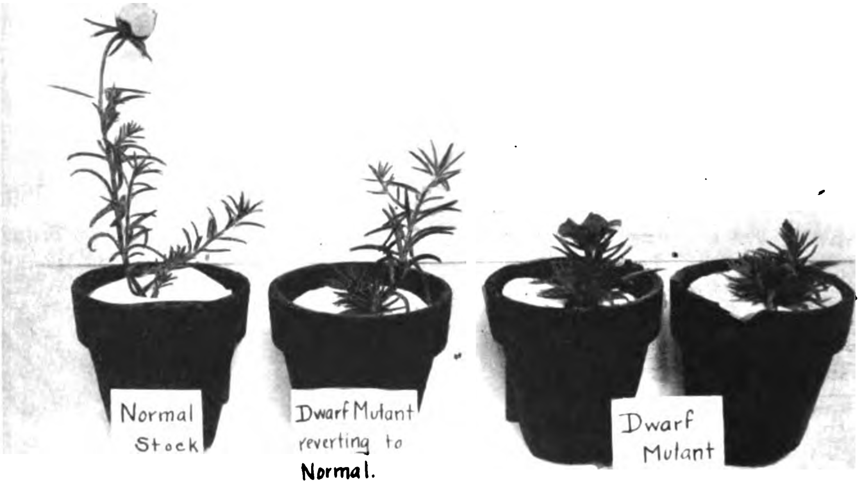

FIGURE 1.—Portulaca. Normal, dwarfs and dwarf reverting to normal

the dwarf plant which inhibits color in its stem though not inhibiting color in its flowers.

Occasionally in the offspring of dwarfs, a few individuals have appeared of an intermediate type which have been called "tall dwarfs." These have pale stems for the most part and longer internodes than typical dwarfs but are not to be confused with normals. Their inheritance has not been investigated in crosses but so far as tested they breed true to the intermediate character when selfed.

Inheritance of dwarf habit

In inheritance the dwarf habit is transmitted as a simple Mendelian recessive. Table 1 shows the result of selfing dwarf plants. All of the 2653 offspring thus obtained are dwarfs with the exception of 14 individuals (0.5 percent) which are normals. These exceptions in cases where the flowers were unguarded against insect visitation might be attributed to chance out-crossing. It seems likely that these as well as the others may better be accounted for by reversions to be discussed later. The normal plant in pedigree 16149 was found to be heterozygous, giving when selfed 29 normals to 15 dwarfs, a fact which would be expected with the assumption of either out-crossing or reversion, but would be less likely from a chance mixture of seed. The unguarded plants listed in these tables were grown in a screened greenhouse and, probably for this reason, gave little evidence of out-crossing when unguarded.

Table 2 shows the result of pollinating normals by dwarfs and the reciprocals. All the offspring are normal. In table 3 are given the numbers of normals and dwarfs in the F_2 offspring. The numbers fail to show a perfect 3 to 1 ratio, being more nearly in the proportion of 4:1. The fact that, in general, mutants tend to be less viable than normals, might account for the reduced number of dwarfs in the F_2 pedigrees. Individual pedigrees however show a considerable variation from expectation.

Table 4 includes the offspring of the F_1 plants back-crossed to the recessive dwarf type. Here again the individual pedigrees are not all as should be expected. Although the two with small numbers can be classed as 1:1 ratios the other two with larger numbers appear to form more nearly a 2:1 ratio. It is probably a curious coincidence only that the two F_1 plants, 16124 (5) and 1692 (4), which when back-crossed gave good 1:1 ratios, gave when selfed, ratios of roughly 4:1 and 9:1 (cf. table 3). This latter pedigree showed the greatest departure from a 3:1 ratio of any in the table.

TABLE 1

Dwarf plants selfed

Record year	Pedigree number	Parent	Pollination	Normal	Dwarf
1916	1560	13206	Guarded		72
1917	16120	1560 (61)	Guarded		50
	16123	1560 (63)	Guarded		23
	16130	1560 (64)	Guarded		6
	16134	1560 (64)	Guarded		11
	16135	1560 (65)	Guarded		61
	16137	1560 (65)	Guarded		18
	16140	1560 (65)	Guarded		85
	16145	1560 (70)	Guarded		25
	16147	1560 (71)	Guarded		24
	16149	1560 (72)	Guarded	1	32
	16152	1560 (74)	Guarded		17
	16157	1560 (78)	Guarded		41
	16160	1560 (79)	Guarded		37
	16165	1560 (126)	Guarded		52
	16170	1560 (126)	Guarded		40
	16178	1560 (138)	Guarded		26
	16184	1560 (140)	Guarded		31
	16191	1560 (142)	Guarded		41
	16205	1560 (145)	Guarded		46
	16220	1560 (172)	Guarded		70
1918	1764	16120 (1)	Unguarded	1	37
	1768	16120 (4)	Unguarded	1	85
	1793	16123 (2)	Guarded		37
	1794	16123 (3)	Guarded		61
	1795	16123 (3)	Unguarded		54
	1769	16140 (1)	Unguarded		90
	1770	16140 (2)	Unguarded		44
	1773	16140 (4)	Unguarded	1	146
	1792	16181 (3)	Unguarded		30
	1796	16205 (2)	Unguarded	1	127
	1797	16205 (3)	Unguarded	1	57
1919	1899	16140 (5)	Unguarded		40
	1898	16165 (3)	Unguarded		394
	1890	1742 (3)	Guarded		18
	1892	1742 (7)	Guarded		3
	184	1744 (3)	Guarded		23
	1895	1745 (4)	Guarded		29
	1851	1746 (4)	Guarded	1	7
	1852	1746 (5)	Guarded		17
	1853	1746 (6)	Guarded		9
	1875	1749 (1)	Guarded		12
	1876	1749 (2)	Guarded		9
	1897	1751 (3)	Guarded	3	204
	18102	1752 (2)	Guarded	4	196
	18103	1757 (1)	Guarded		43
	18104	1758 (1)	Guarded		43
	18105	1759 (1)	Guarded		30
Totals—Guarded capsules only				9	1549
Unguarded capsules only				5	1104
Guarded and unguarded capsules				14	2653

TABLE 2
(*Normal* × *dwarf*) F_1

Cross	Number of pedigrees			Normals	Dwarfs
	1917	1918	1919		
Normals × dwarfs	23	2	1	590	0
Dwarfs × normals	6			112	0
Totals	29	2	1	702	0

TABLE 3
(*Normal* × *dwarf*) F_2

Pedigree number	Parent	Pollination	Original cross Dwarf × normal		Original cross Normal × dwarf		Grand totals	
			Normal	Dwarf	Normal	Dwarf	Normal	Dwarf
1742	16124 (2)	Unguarded	77	22				
1743	16124 (5)	Unguarded	82	22				
1744	16146 (2)	Unguarded	25	14				
1745	16148 (2)	Guarded	99	21				
1746	1625 (4)	Guarded			63	15		
1747	1652 (2)	Unguarded			34	13		
1750	1693 (4)	Unguarded			89	13		
1749	16104 (2)	Guarded			48	15		
1866	1692 (4)	Guarded			94	10		
1842	1738 (1)	Guarded			95	21		
1849	1740 (1)	Guarded			81	28		
1850	1740 (2)	Guarded			90	18		
1886	1741 (1)	Guarded			78	19		
Totals—Guarded capsules only			99	21	549	126	648	147± 8.23
Unguarded capsules only			184	58	123	26	307	84± 5.77
Guarded and unguarded capsules			283	79±5.56	672	152±8.38	955	231±10.06
Calculated ratios			*271.5*	*90.5*	*618*	*206*	*889.5*	*296.5*

TABLE 4
F_1 × *dwarf recessive*

Pedigree number	Parent	(Dwarf × normal) F_1 × dwarf		(Normal × dwarf) F_1 × dwarf		Totals	
		Normal	Dwarf	Normal	Dwarf	Normal	Dwarf
1758	1625 (5) × 1560 (72)			44	23±2.76		
1759	1692 (4) × 1560 (76)			9	7±1.35		
1756	16124 (5) × 1560 (76)	15	15±1.85				
1757	16159 (6) × 1560 (60)	25	10±1.69				
Totals		40	25±2.72	53	30 ±3.07	93	55±4.10
Calculated ratios		*32.5*	*32.5*	*41.5*	*41.5*	*74*	*74*

From the foregoing tabulations one would feel satisfied in concluding that the dwarf character in Portulaca is a simple Mendelian character recessive to the dominant normal habit of growth. Recessive characters when they appear should be homozygous for their determining factors and in consequence dwarfs when selfed should give rise only to dwarfs and never to normal plants. The number of exceptions in our cultures (0.5 percent) though small, we cannot believe were due entirely to experimental errors. Their significance may be clearer after a discussion of the phenomenon of vegetative reversions.

VEGETATIVE REVERSIONS

The most interesting peculiarity of the mutant under discussion is the fact that some of the dwarf plants give rise to branches normal in appearance. Such a reverting dwarf is shown in figure 1. The reverting branches have long internodes and red stems and are otherwise indistinguishable in appearance from pure normals.

Frequency of occurrence of reversions

Reverting branches may occur apparently at any stage in the development of the plant. They have been found occurring late in the season on large dwarf plants and also at so early a stage that the specimen would have been classified as a normal if the small dwarf axis had not been discovered at the base of the plant. It seems best in computing their frequency to take into consideration only a series that was sown and kept under observation for this purpose. The pedigrees in table 5 were grown to discover if increasing the amount of bone meal in the nutrient would increase or decrease the frequency of reverting branches. No effect of the treatment upon the occurrence of reverting branches could be observed. The data accordingly can be combined as shown in table 6 to give the proportion of dwarfs with reverting branches (called "reverters") to typical dwarfs. Of the 1294 dwarfs in this table, 16, or 1.23 percent, were reverters. Reverters have been classed with dwarfs in table 6 as in previous tables. The proportion of reverters and of normals is higher in certain pedigrees than in others. The data are not sufficient however to indicate whether an increased tendency toward reversions and normals actually exists in these cases. Reversion may probably take place at any stage in the development of the zygote. It might even be expected at the formation of the gametes. If this is true we

TABLE 5

Dwarf plants selfed, reverter test

Pedigree number	Parent	Pollination		Pure sand A	Sand B	Soil C	Soil + 50 D	Soil + 100 E	Soil + 150 F	Soil + 200 G	Soil + 250 H	Soil + 300 I	Soil + 400 J	Soil + 500 K	Total, A–K incl.
True dwarfs 1897	1751 (3)	Guarded	Dwf	20	19	19	19	19	19	20	20	20	17	10	202
			Rev			1							1		2
			Nor				1	1	1						3
			dd		1								2		3
1898	16165 (3)	Unguarded	Dwf	40	40	39	39	38	38	38	40	32	29	16	389
			Rev						1	1		1		2	5
			Nor												
			dd			1	1	2	1	1		7	11	22	46
1899	16140 (5)	Unguarded	Dwf		15	15								10	40
			Rev												
			Nor												
			dd											5	5
18102	1752 (2)	Guarded	Dwf	19	18	16	18	20	18	19	20	20	14	6	188
			Rev		2	1	1		2	1			1		8
			Nor	1		1	1						1		4
			dd			2							4	14	20
18103	1757 (1)	Guarded	Dwf		15	15								13	43
			Rev												
			Nor												
			dd											2	2
18104	1758 (1)	Guarded	Dwf		14	15								13	42
			Rev		1										1
			Nor												
			dd											2	2
18105	1759 (1)	Guarded	Dwf		10	10								10	30
			Rev												
			Nor												
			dd												
Tall dwarfs 1896	16123 (6)	Guarded	Dwf	20	20	20	20	20	20	20	19	10			169
			Rev												
			Nor												
			dd								1				1
18100	1743 (1)	Guarded	Dwf		20	20		20		20		20			100
			Rev												
			Nor												
			dd												
18101	1743 (2)	Guarded	Dwf		25	25								25	75
			Rev												
			Nor												
			dd												

Explanation.—The plants were grown in different substrata indicated by letters in heading. A is sand cleaned and watered with distilled water. B is sand watered with tap water. C is potting soil. D to K is potting soil plus bone meal. In each case the amounts of bone meal in grams noted in the table were added to about 6 kilos of soil. Dead, unrecordable plants are listed as "dd."

should expect plants that have reverted at or before the seedling stage to be indistinguishable in appearance from normals. Normals in these pedigrees therefore may be considered as early reverters. If so and we include the 7 normals with the 16 obvious reverters of table 6 we have 23 reversions out of 1301 plants or 1.77 percent.

TABLE 6

Dwarf plants selfed; reverter test

Pedigree number	Parent	Pollination	Dwarf	Reverter	Normal	Total
1897	1751 (3)	Guarded	202	2	3	207
1898	16165 (3)	Unguarded	389	5		394
1899	16140 (5)	Unguarded	40			40
18102	1752 (2)	Guarded	188	8	4	200
18103	1757 (1)	Guarded	43			43
18104	1758 (1)	Guarded	42	1		43
18105	1759 (1)	Guarded	30			30
1896	16123 (6)	Guarded	169			169
18100	1743 (1)	Guarded	100			100
18101	1743 (2)	Guarded	75			75
Total plants recorded			1278	16	7	1301
Percents			98.23	1.23	0.54	100.00

Genetic constitution of reverting branches

The genetic constitution of reverting branches can be discovered (1) by selfing individual flowers of reverting branches, and (2) by back-crossing them to typical dwarfs and normals.

The result of selfing flowers from reverting branches is shown in table 7. A summary of dwarfs and normals in the offspring shows a close agreement with a 3 : 1 ratio and indicates that the reverting branch is heterozygous for the dwarf character. The first two figures in the pedigree number represent the year in which the seed was harvested. It will be noted that pedigrees 16128, 16181, and 16186 are from the same plants respectively as 1778, 1780, and 1781. These latter pedigrees were from cuttings from reverting branches, rooted and kept growing to the second year. The three reverting branches of these plants selfed in 1916 gave a ratio of 95 : 20 which in numbers per 4 is 3.30 : 0.70 ± 0.109 with a difference from the expectation of 2.75 times its probable error, while cuttings from the same reverting branches a year later gave a ratio of 255 : 75 which in numbers per 4 is 3.09 : 0.91 ± 0.064 with a difference from expectation of only 1.41 times its probable error. We may conclude in consequence that in their growth for upwards of a year these reverting branches had not undergone any change that would increase their tendency to throw normals.

TABLE 7

Reverting branches selfed

Pedigree number	Parent	Pollination	Reverting branch	
			Normal	Dwarf
16128	1560 (64)	Guarded	26	4
16181	1560 (138)	Guarded	15	2
16186	1560 (140)	Guarded	54	14
16193	1560 (142)	Guarded	59	19
16199	1560 (142)	Guarded	46	9
16206	1560 (145)	Guarded	34	6
16208	1560 (145)	Guarded	25	20
16210	1560 (145)	Guarded	50	16
1760	16120 (1)	Guarded	84	37
1761	16120 (1)	Unguarded	66	24
1762	16120 (1)	Unguarded	35	9
1765	16120 (2)	Unguarded	25	6
1767	16120 (4)	Unguarded	16	6
1771	16140 (2)	Guarded	61	27
1772	16140 (2)	Unguarded	53	17
1774	16140 (4)	Guarded	48	12
1775	16165 (1)	Guarded	32	5
1776	16165 (1)	Unguarded	104	38
1778	1560 (64)	Unguarded	91	30
1780	1560 (138)	Unguarded	95	14
1781	1560 (140)	Unguarded	69	31
1791	16181 (3)	Unguarded	146	36
Total—Guarded capsules only			534	171± 7.75
Unguarded capsules only			700	211± 8.81
Guarded and unguarded capsules			1234	382±11.74
Calculated values			*1212*	*404*

In table 8 are summarized the results of selfing reverting branches and dwarf branches from the same plants. The dwarf branches breed true to the dwarf character to the same extent shown by dwarf plants that have not reverted. It is for this reason that dwarf branches of reverters have been included in table 1.

The normal plants obtained by selfing reverting branches have themselves been tested by being selfed, with the results shown in table 9 (A).

TABLE 8

Reverting branches and dwarf branches from same plant selfed

Parent	Reverting branch		Dwarf branch	
	Normal	Dwarf	Normal	Dwarf
1560 (64)	117	34	0	17
1560 (138)	110	16	0	26
1560 (140)	123	45	0	31
1560 (142)	105	28	0	41
1560 (145)	109	42	0	46
16120 (1)	185	70	1	37
16120 (4)	16	6	1	85
16140 (2)	114	44	0	44
16140 (4)	48	12	1	146
16181 (3)	146	36	0	30
Totals	1073	333±10.95	3	503
Calculated values	*1054.5*	*351.5*		

TABLE 9

(A) Normals from reverting branch selfed

Pedigree number	Parent	Pollination	Segregating pedigrees		Uniform pedigrees	
			Normal	Dwarf	Normal	Dwarf
1782	16128 (1)	Guarded	79	12		
1783	16128 (2)	Guarded	43	13		
1784	16128 (3)	Guarded	101	18		
1789	16181 (1)	Guarded	37	5		
1790	16181 (2)	Unguarded	45	24		
1785	16186 (1)	Guarded			33	0
1786	16186 (2)	Guarded	38	11		
1787	16186 (3)	Unguarded	162	50		
1788	16186 (4)	Unguarded	130	34		
Totals—Guarded capsules only			298	59	33	0
Unguarded capsules only			337	108		
Guarded and unguarded capsules			635	167±8.27	33	0
Calculated values			*601.5*	*200.5*		

(B) (Normal × reverting branch) F_2

Pedigree number	Parent	Pollination	Segregating pedigrees		Uniform pedigrees	
			Normal	Dwarf	Normal	Dwarf
1753	162 (2)	Guarded			74	0
1754	162 (3)	Guarded	83	17		
1755	1648 (1)	Unguarded			72	0
1751	16102 (4)	Unguarded	68	13		
1752	16114 (1)	Unguarded	112	19		
Totals—Guarded capsules only			83	17	74	0
Unguarded capsules only			180	32	72	0
Guarded and unguarded capsules			263	49±5.17	146	0
Calculated values			*234.75*	*78.25*		

One would expect more of these normals to be heterozygous than homozygous for the normal character. This expectation is realized, although not in the exact proportion of two heterozygous to one homozygous individual which a larger series of tests might more nearly approximate.

The second method of testing the genetic constitution of reverting branches is to cross them with each member of the allelomorphic pair. In all, 10 pedigrees were secured by crossing reverting branches with dominant normals. The 309 offspring in the F_1 generation were all normals. The expectation is that half of the normals would be homozygous normals and half would be normals heterozygous for dwarfing. This expectation is well satisfied as shown in table 9 (B), inasmuch as three are segregating pedigrees and two are uniform.

TABLE 10

Reverting branch × dwarf recessive

Pedigree number	Parents	Normal	Dwarf
·16131	1560 (64) × 1560 (64)	1	1
16132	1560 (64) × 1560 (64)	3	0
16190	1560 (142) × 1560 (142)	4	9
1763	16120 (1) × 1560 (76)	22	19
1766	16120 (2) × 1560 (76)	10	6
1777	1560 (64) × 1560 (72)	41	46
1779	1560 (64) × 1560 (72)	7	9
Totals		88	90±4.50
Calculated values		*89*	*89*

A more convenient method of testing is to back-cross with the recessive dwarfs. The results are shown in table 10. The expectation of a 1:1 ratio is satisfied in that in the offspring 88 normals were obtained to 90 dwarfs. In all the crosses the reverting branches were used as the seed parents except 16131 which is the reciprocal of 16132. These two pedigrees and 16190 are crosses between reverting and dwarf branches of the same plant.

In table 11 are given summaries of the 1918 field tests of reverting branches of 3 plants which were both selfed and crossed with the dwarf recessive. This detailed analysis leaves no doubt that reverting branches are dominants heterozygous for the recessive factor.

TABLE 11

Reverting branch selfed and back-crossed to dwarf

Pedigree numbers	Parent	Reverting branch selfed		Reverting branch × dwarf	
		Normal	Dwarf	Normal	Dwarf
1760, 1761, 1762	16120 (1)	185	70		
1763	16120 (1) × 1560 (76)			22	19
1765	16120 (2)	25	6		
1766	16120 (2) × 1560 (76)			10	6
1778	1560 (64)	91	30		
1777 and 1779	1560 (64) × 1560 (72)			48	55
Totals		301	106±5.89	80	80±4.26
Calculated values		*305.25*	*101.75*	*80*	*80*

DISCUSSION OF RESULTS

The dwarf mutant of Portulaca is not peculiar in its inheritance. Like many dwarfs it is a recessive character. Like many other recessive mutants, the number of dwarfs runs somewhat below expectation in the F_2 generation. The chief interest in this form lies in the production of branches with the dominant habit of growth on otherwise recessive plants. Such reverting branches come under the category of bud sports or vegetative mutations. The appearance of bud variations is not a rare phenomenon in Portulaca, many of them being apparently in the nature of sectorial chimeras. The mutation responsible for the reverting branches apparently affects a single one of the paired recessive genes, changing it to the dominant allelomorph. In consequence the branch is heterozygous. This was the case in the dozen mutant branches tested, as shown in table 7. Extensive tests might ultimately discover a reverting branch homozygous for the dominant character. Such a condition would be expected, however, only after both of the recessive genes for dwarfing had reverted to normal, either simultaneously, or at different stages in development. Since heterozygous and homozygous branches are alike in appearance, their distinction can only be made by a breeding test. It seems not improbable that any single cell of the dwarf plant is liable to mutate to normal in one of the two paired genes. It is difficult to calculate what percentage of the dwarf cells mutate in this manner. If the 0.5 percent normals appearing in dwarf pedigrees could be assumed to come from mutating gametes we might calculate that 0.5 percent of the zygotes had the mutant gene or that 0.25 percent of the gametes themselves had mutated. Such an assumption, however, appears unwarranted since it is probable that some of the normals in dwarf pedigrees are to be considered reversions that have taken place at an early seedling stage. However, it can be assumed that certainly not over 0.25 percent or 1 in 400 of the gametes are liable to mutate. The percentage of mutated gametes is probably much smaller.

It may seem strange that the mutations from dwarf to normal seems relatively common while the reverse mutation, from normal to dwarf, has not been observed aside from our original dwarf of 1914. This fact might lead to the conclusion that mutation from a recessive to a dominant gene in this specific instance is more frequent than the mutation from the dominant to the recessive member of the pair. A brief consideration will show that such a conclusion would not be warranted from the data at hand. To be evident, dwarf mutations would have to exist in the homozygous (*aa*) condition. Using our figures above and assuming that the genes for normal mutate to dwarf as often as those for dwarf mutate to normal, viz., once in 400 times, in the formation of the gametes, we should require 400 times 400 or 160,000 gametes in order to have a single chance of obtaining a zygote with both determiners for dwarfing. One at the most out of some 80,000 offspring of selfed normal plants therefore should be dwarf (*aa*), and one at the most out of 200 normal plants should be heterozygous (*Aa*) and when selfed throw a 3:1 ratio of normals and dwarfs. Moreover, 1 out of 400 plants from the cross of normal by dwarf shown in table 2 should be dwarf, on this assumption. We have grown over 5300 offspring from lines with normal habit, including 109 selfed parents and 702 offspring from the cross of normal by dwarf and have never seen dwarf plants in the species, except those which could be traced back to the dwarf mutant found in 1914.

In table 12 are tabulated the chances (C) for the appearance of dominant and recessive mutations corresponding to various percentages (P) of mutated gametes. Formulae are given and their application shown by a few examples. In our figures above we assumed that all the 0.5 percent normals which appeared in dwarf pedigrees came from mutations in 0.25 percent of the gametes. The figure would probably still be much too high if it were a fifth as large or 0.05 percent. On the latter assumption, dwarfs would be expected to appear only once in 2,000,000 times from mutations in the gametes alone. Whatever figure is taken, it is obvious from the table that even though the appearance of a dominant mutant may be relatively common, the opposite recessive mutant will be found extremely rare although the mutations in the individual chromosomes may be equally frequent in the two directions.

If vegetative mutation from normals to dwarfs occurred with any frequency, they might be expected to appear as bud sports on heterozygous normal plants or on reverting branches. They have been looked for in the large number of heterozygous normals in our pedigrees but have not been discovered. Due to their slowness of growth, however, it is doubtful if they would have an opportunity of showing themselves even if such mutative changes should take place in the vegetative tissues involving a bud. A short dwarf branch on a vigorous normal plant would likely escape notice. Moreover, it is likely that a dwarf mutant bud would be entirely prevented from growing by the development of the more vigorous normal buds. So far as the obvious evidence goes, mutations from the recessive dwarf to the dominant normal condition is not extremely rare in this species while the mutation from the dominant normal to the recessive dwarf is a phenomenon that has been observed only once.

TABLE 12

Percentage of mutant gametes	Chances of heterozygous mutant (*Aa*) from *AA* or *aa* selfed	Chances of homozygous mutant (*AA* or *aa*) from cross (*AA* × *aa*)	Chances of *AA* mutant from *aa*, or *aa* mutant from *AA*
P	$C = 1:\frac{50}{P}$	$C = 1:\frac{100}{P}$	$C = 1:\frac{5,000}{P^2}$
0.25	1:200	1:400	1:80,000
0.125	1:400	1:800	1:320,000
0.05	1:1,000	1:2,000	1:2,000,000
0.025	1:2,000	1:4,000	1:8,000,000

It is not our purpose at the present time to enter into any detailed discussion of the subject of vegetative mutations in Portulaca. EAST'S (1917) paper on bud variations may be referred to in passing. It is agreed that bud variations come more frequently in hybrids due probably to the fact that in heterozygotes a mutation in only a single gene would be required for the appearance of a recessive which is the commonest type of vegetative mutations. The fact that the mutation under observation takes place with apparent frequency in one direction and not in the other may be due in part to the chances against the appearance of both the recessive genes in the same zygote.

SUMMARY

1. A dwarf mutation of *Portulaca grandiflora*, characterized by short internodes, is described and figured.

2. The dwarf habit of growth is inherited as a simple Mendelian character recessive to the dominant normal habit.

3. About 1.25 percent of the recessive dwarf plants produce reverting branches with the dominant normal habit of growth.

4. Occasional normals found in offspring of selfed dwarfs are probably due to reversions bringing about normal gametes or to reversions in the early development of the zygote.

5. Dwarf branches from reverting plants when selfed breed as true as typical dwarfs.

6. Reverting branches from otherwise dwarf plants are heterozygous and give 3:1 ratios, when selfed, and 1:1 ratios, when back-crossed to dwarfs.

7. The reverting branches produced by dwarfs are classed as dominant vegetative mutations.

LITERATURE CITED

EAST, E. M., 1917 The bearing of some general biological facts on bud-variation. Amer. Nat. 51: 129–143.

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BEARING ON HEREDITY AND
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CALCULATING ANCESTRAL INFLUENCE IN MAN: A MATHEMATICAL MEASURE OF THE FACTS OF BISEXUAL HEREDITY[1]

HARRY H. LAUGHLIN

Eugenics Record Office, Cold Spring Harbor, Long Island, New York

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According to the common method of calculating ancestral influence in man, the germ-plasm is considered as an indefinitely dilutable substance contributed equally by the father and mother in making up the hereditary constitution of the offspring. Barring special phenomena, the several direct ancestors in each generation are, among them, quite properly accredited with the possession, patently or latently, of all of the traits of a particular offspring. In calculating the influence of a given ancestor in contributing toward the whole body of these traits in the offspring, the measure is a quotient determined by dividing unity by the number of direct ancestors in the given generation. Thus, the ancestral influence of one of the four grandparents is commonly said to be one-fourth, that of one of the eight great-grandparents one-eighth, and so on.

Sir FRANCIS GALTON, after a very careful study of the inheritance of human stature, proposed a modification of this orthodox notion of ancestral influence. His scheme is more in consonance with many of the observed facts than is the common method of dividing unity by the number of direct ancestors in the particular generation. His rule, or GALTON'S Law as it is sometimes called, ascribes one-half of all ancestral influence to the two parents (each parent one-half of 50 percent, or 25 percent), one-fourth to the four grandparents (each grandparent one-fourth of 25 percent, or 6.25 percent), one-eighth to the eight great-grandparents (each great-grandparent one-eighth of 12.5 percent, or 1.5625 percent). Later KARL PEARSON made some mathematical refinements of this scheme. But the study of pedigrees is so replete with cases showing the segregation and re-combination of ancestral traits that, even when traits are massed and averaged, neither the common quotients of the genealogists nor the generalizations of GALTON or PEARSON suffice to explain the pedigree-facts, nor does either scheme square with the cytological evidence in the case.

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[1] Read before the NATIONAL ACADEMY OF SCIENCES, November 10, 1919, at New Haven, Connecticut.

With the rise of Mendelian investigations and the discovery of the close co-ordination between Mendelian pedigree-findings and the cytological evidence of the mechanism of heredity, there was founded the modern science of heredity, which is much more generally consistent with the observed facts than are any of the older systems of gauging natural inheritance.

By ancestral influence is meant the contribution of definite hereditary mental, physical and moral qualities, by a given ancestor to a given descendant. Pedigree-studies have set forth in man, as in many other organisms, rules of inheritance of many specific traits or qualities. Cytological investigations have shown how chromosomes duplicate, segregate and recombine (sometimes crossing-over and showing other special phenomena) in the course of gametogenesis, fertilization and development.

Neither the human nor any other germ-plasm is indefinitely dilutable or divisible, as many genealogists have assumed, but the dilutability and divisibility of the germ-plasm are limited by the number of chromosomes characteristic of the gametes of the particular species. In the human zygote two gametes, each with 12 chromosomes, unite.[2] This means that, barring crossing-over, mutation, and other special phenomena, normally the human germ-plasm, and consequently all hereditary human qualities, that is, the "blood," is divisible only into 24 uneven units corresponding to the 24 zygotic chromosomes.

It is well demonstrated that the chromosomes are composed of genes or the determiners of natural inheritance. Each chromosome is a gene-radical or linkage-group of trait determiners. If then it is sought to measure the influence of a given ancestor upon the hereditary traits of a given propositus, it is necessary to develop a series of mathematical formulae which measure the probabilities of definite situations happening in reference to the transmission of chromosomes from the given ancestor to the given propositus.

In developing such formulae the following factors must be taken into consideration:

First. Whether the species in question is bisexual.

---

[2] There is still disagreement among cytologists concerning the number of chromosomes in man. VON WINIWARTER (1912) concludes that the zygotic number of chromosomes in the human male is 47, in the human female 48, while WEIMAN (1917) concludes that the zygotic or diploid number of chromosomes is 24, of which 2 are the XY idiochromosomes. When this problem is settled beyond peradventure, the correct number can be used in the accompanying formulae, which are here stated in general terms. For a history of the cytological study of the chromosome number in man see the excellent analytical bibliography given by ETHEL BROWNE HARVEY in Journal of Morphology **34**: 47–49, June, 1920.

Second. Whether in the particular species the male or the female is the heterozygous sex.

Third. Due provision must be made for sex-linked traits.

Fourth. The number of chromosomes, both gametic and zygotic, characteristic of the species, must be known.

Fifth. For the present the individual chromosomes will not be weighted, but simply counted. Doubtless the ontogenetic working out of each chromosome is very specific both qualitatively and quantitatively. When ultimately genetical and cytological research locate in definite chromosomes the genes for definite traits, then the several chromosomes can be weighted, and due provision for their differential values properly made in the formulae for measuring the several features of ancestral influence.

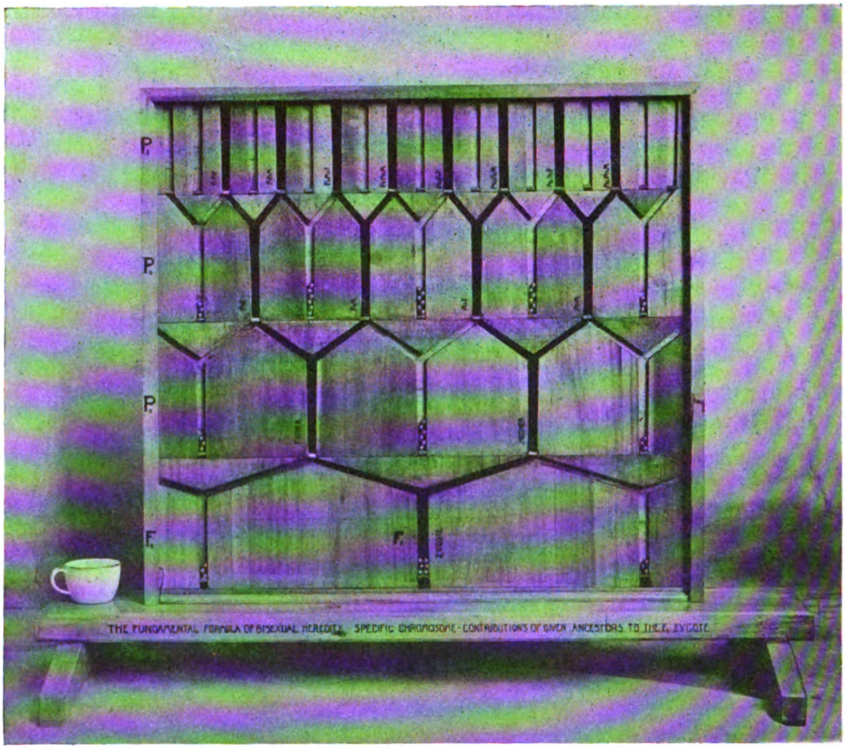
Sixth. Due cognizance must be taken of the possibility of mutation, crossing-over, non-disjunction and other special chromosomal phenomena occurring in the course of the descent of chromosomes from a given ancestor to the propositus. In the accompanying formulae such phenomena are not provided for, because their rates of occurrence are not yet measurable in man, but when one by one their mathematical measures are attained, each such measure can be woven into the foundation formulae here given. The present formulae will not be upset by such corrections, but simply modified in the direction of greater accuracy.

Seventh. It must be considered that in a given gamete a definite chromosome may have been derived from either the paternal or the maternal source. This is especially important in cases of zygotes of mixed or mongrel blood.

Eighth. In certain situations it will be necessary to specify whether a given trait is dominant or recessive to its allelomorph.

Finally, all formulae must be stated in general terms, so that they may apply with equal accuracy to any species of plant or animal concerning which chromosomal data are at hand.

Applying these principles to man, the accompanying charts set forth in pedigree-fashion and measure mathematically a few selected definite features of the hereditary influence of each particular ancestor taken as a type, upon the propositus or F_1 zygote. These calculations are based upon the following data and conditions: (a) that the gametic number of human chomosomes is 12, the zygotic or somatic number in each sex 24; (b) that the male is heterozygous (XY) and the female homozygous (XX) in reference to sex; (c) that all of the purely hereditary qualities are carried by the several chromosomes; and (d) that in the first or foundation formulae no provision will be made for mutation, crossing-over, non-disjunction and other special phenomena.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THE FUNDAMENTAL FORMULA OF BISEXUAL HEREDITY. SPECIFIC CHROMOSOME-CONTRIBUTIONS OF GIVEN ANCESTORS TO THE F_1 ZYGOTE

FIGURE 1.—Mechanism showing the essential features of segregation and recombination in the transmission of chromosomes from ancestors to descendants.

In the case here pictured, as shown by the location of the balls, the descent of chromosomes is traced in a bisexual species characterized by three pairs of chromosomes. Into each of the eight slots representing the eight ancestors of the P_3 generation, six balls were fed (through openings in the top of the machine not shown in the figure), to represent the six somatic chromosomes (three pairs). If the balls are given a distinct color or stamp for each of these eight ancestors, the tracing of the individual balls becomes clearer. The slots are constructed so that the balls, when fed into them, will settle alternately to the right and left, or *vice versa*, depending upon the chance settling of the first ball. The dividing wedge below the slot is located with its apex on the mid-line of the slot, so that when the slide is withdrawn from the back the balls pass alternately to the right and left, or *vice versa*, depending upon the particular system of stacking. Thus in the operation of the machine the segregation and recombination of balls follows the same mathematical rules which (barring crossing over, non-disjunction, mutation, special systems of selection, and other special phenomena) govern the descent of chromosomes in normal bisexual heredity. The fundamental formulae given in the accompanying paper may be demonstrated by its use.

This machine is 33 inches by 35½ inches in size, not including the base. Its face is covered with glass $\frac{3}{16}$ inch thick. The dark line traces ancestral descent for the F_1 zygote. At the bottom of each slot there is a wooden stop which withdraws from the back, and below each *cul-de-sac* slot there is a tin chute which opens to the rear, and enables the operator conveniently to remove the balls. The three lowest slots also open into similar chutes.

Assuming the above conditions (and assuming that, until future research results in weighting ontogenetic values of specific chromosomes, each chromosome carries about the same number of genes or determiners for traits as each of the other 11 chromosomes), we find that we should speak of "blood" or ancestral influence not alone in terms of quotients obtained by dividing unity by the number of direct ancestors of the particular generation, although this has a certain genealogical value, but should measure ancestral influence by the probability that a given number of chromosomes or gene-radicals, according to the demonstrated laws of heredity, will descend from the given ancestors to the particular propositus.

Under the old scheme, each one of the 16 great-great-grandparents was thought of as contributing one-sixteenth of the "blood" possessed by the descendants of the F_1 generation. The average contribution in chromosome-number, (barring the sex-chromosome, which follows much more restricted paths in the family-tree), characteristic of such an ancestor, is 1.375. This, of course, is one-sixteenth of the eleven equivalent chromosome-pairs. But this is not a constant contribution. The range of chromosome-contribution by members of the great-great-grandparental generation, barring those individuals in the X- or Y-trails, varies from none to eleven chromosomes. Each particular case must, of course, be analyzed by pedigree-methods to determine what the actual contribution has been in the case of an individual already born. Actually, (barring special phenomena), each parent or ancestor contributes either none or a definite unit number of chromosomes. This number may be as high as 12 in man, or in any species as high as the gametic number of chromosomes. A fractional number following formula A on the pedigree-charts (plates 1 and 2) measures an average calculated in reference to the person, taken as a type, occupying the given position in the pedigree.

It is evident that if we wish a more accurate measure of ancestral influence than is given by the ordinary methods, not only must the average number of chromosomes contributed by a given ancestor, taken as a type, be calculated, but also the possible range in number of chromosomes contributed must be stated.

But besides those worked out on the accompanying pedigree-charts, there are many other mathematically measurable features of ancestral influence. For instance, the probability of transmitting from a given ancestor to the F_1 zygote not only a definite number but a definite combination of specific chromosomes, also the probability of paternity or maternity of descent of particular chromosomes to the ancestor who passes them on, can be formulated.

Ultimately the biological analysis of a human pedigree will resolve itself into tracing specific chromosomes and parts of chromosomes as the physical determiners of traits, from the propositus back through the family-tree. Thus if, as seems probable, there are but 12 chromosomes in each human gamete, then each half-pedigree, regardless of how many individuals there are in the ancestral generation, can contribute but 12 chromosomes. For example, in the paternal and maternal half-pedigrees, in the P_6 generation there are, in each, sixteen direct ancestors; then (barring crossing-over) at least four of these ancestors must be left out entirely so far as chromosomal contribution, and consequently ancestral influence, is concerned. Of course each ancestor is physiologically necessary as a nurse for the germ-plasm, even though in the course of descent all of the chromosomes of a particular ancestor be shunted in the processes of gametogenesis. The location in the pedigree, of the ancestor supplying the gamete which presents a crossing-over in one of the chromosomes, may be established when the knowledge of linkage and the linear geography of chromosomes becomes more exact. Thus until chromosomes are actually weighted as to their relative ontogenetic influence, we must divide "blood" not into halves, quarters, eighths, sixteenths and so on, by the usual genealogical method, but into twenty-four uneven and individual parts. While it is thus proper for genealogical purposes to speak of an individual as fifteen-sixteenths white and one-sixteenth negro, to be biologically correct reference should be to an individual carrying so many (and which) of the total of twenty-four chromosomes from white ancestors and so many from black. It is perfectly proper to speak of a human individual as seven twenty-fourths or nineteen twenty-fourths of one race, provided the twenty-four units are conceived of as individual and, possibly, very unequal elements. With the weighting of chromosomes and the evaluation of fractional chromosomes in crossing-over, the fractions measuring "blood" or ancestral influence will possess no standard denominators, unless perchance such denominator be the ultimate number of human genes.

In any particular zygote there is of course no chance that a given ancestor will contribute anything except what he has actually contributed. The ratios shown on the accompanying pedigree-charts are measures of probability in reference to events which have not yet happened, that is, measures applicable to descendants not yet born. The averages refer not to specific individuals, but to the occupant of a definite position in the pedigree, taken as a type. The ratio of an accompanying formula may be viewed also in the light of an even or a safe bet or probability, if we be ignorant of the facts in a specific case, that a given ancestor contributed thus and so from his germ-plasm to the given zygote.

Biologically the formulae here given are as sound as current biological knowledge. They may be modified and developed in accordance with the advance of biological learning. They are equally as applicable to any normally bisexual species of plant or animal as to man.

Mathematically the accompanying formulae are built upon the following elementary principles of (a) probability, and (b) combination.

(a) *Probability*

1. The certainty that an event will occur is measured by the quotient $1:1$.

2. The impossibility of an event occurring is measured by the quotients $0:\infty$ or $1:\infty$.

3. If out of n possible happenings, there are no conditions favoring the happening of one possibility over another, the probability that a given event will occur is $1:n$.

4. If out of n possible happenings, there are no conditions favoring the happening of one possibility over another, the probability that a given event will not occur is $1:\frac{n}{n-1}$.

5. The probability that two or more events will occur is measured by the product of their respective probability-quotients, provided that between or among the several possibilities there is no definite dependence or interference.

6. The probability that none of two or more independent events will occur is measured by the product of the quotients of probability that each event separately will not occur.

7. The probability that a given complex (of certain independent events happening and certain other independent events not happening) will eventuate, is measured by the continued product of the several quotients of probability that each particular constituent event as chosen will or will not occur.

8. The probability that a single possible event will occur within a given portion of a definite range of possibilities is measured by the sum of the probability-quotients of the separate chances constituting the particular given portion.

9. The probability that a single possible event will not occur within a given portion of a definite range of possibilities is $1:\frac{n}{n-h}$, in which

n = the total number of equal chances in the range.
h = the number of equal chances in the given portion.

(*b*) *Combinations*

c = whole number of combinations possible within n factors taken m at a time.

$$c = \frac{n(n-1)(n-2)\ldots(n-m+1)}{1.2.3\ldots m}.$$

n = whole number of factors.
m = number of factors taken at a time (never greater than n).

FORMULAE FOR CALCULATING ANCESTRAL INFLUENCE IN BISEXUAL SPECIES

The values of formulae number 1, 4, 5, 6, 7, 8, 9 and 10 for each individual to whom the particular formulae apply are given on the accompanying charts showing the ancestral influence in the human male, and in the human female. (Plates 1 and 2.)

1. A = average number of chromosomes passed on by the selected ancestor, taken as a type, to the F_1 zygote, i.e., the propositus.

A = also the average number of chromosome-pairs each of which contributes one member of the diploid or somatic group of chromosomes of the F_1 zygote.

In man the diploid or somatic number of chromosomes is taken tentatively (see explanatory note, page 436) at 24, i.e. 11 equivalent pairs and one XX or XY sex pair.

$A = \frac{n}{2^{(p-1)}} + (C_y$ *or* C_x in case of ancestors from whom an X or a Y chromosome *may* descend.)

See plates 1 and 2 for X- and Y-trails.

2. n = number of equivalent chromosome-pairs (i.e., excluding the XY or XX sex-pair) characteristic of the species. In the case of man n is taken as 11.

3. p = the numerical value of the sub-figure of P used in pedigree-manner to indicate the ancestral or parental generation in relation to the propositus or principal figure of the pedigree. Thus in the great-grandparental generation, P_3, $p = 3$.

4. C_y = the average number of Y-chromosomes contributed to the F_1 zygote by the particular *male* ancestor taken as a type.

$1:C_y$ = the probability that a particular *male* ancestor will contribute the single Y-chromosome found in the F_1 *male* zygote.

The value of $1:C_y$ is always $1:1$, and it is applicable to the *direct male* line only. For an ancestor not in the Y-trail, $1: C_y = 1:\infty$.

This direct paternal line down which the *Y*-chromosome descends is the so-called "Y-trail."

5. C_x = the average number of X-chromosomes contributed to the F_1 zygote by the particular ancestor taken as a type.

$1:C_x$ = the probability that the particular male or female ancestor will contribute an X-chromosome to the F_1 zygote.

The value of C_x is determined by the following rule:

(a) Beginning with the X-chromosomes in the F_1 zygote, without exception (i.e., with the chance of $1:1$) the *single* X-chromosome in the *male* is derived from his *mother*; and of the *two* X-chromosomes in the *female*, *one* is derived from *each parent*.

(b) In the P_1 and all older generations, in the case of a female zygote the chance of the descent of an X-chromosome from the *father* or *mother* is *equal* (i.e., $1:2$), but in the *male* the X-chromosome is always received from the *mother* alone (i.e., the chance is $1:1$).

Therefore, the probability that a given ancestor, taken as a type, will contribute an X-chromosome to the F_1 zygote, (i.e., $1:C_x$), is equal to the product of all probability ratios, made according to rules (a) and (b) above, along the X-trail from the selected ancestor to the P_1 generation inclusive.

Thus $1:C_x$ for the mother's mother's father of a female zygote $= (1:1) . (1:2) . (1:2) = 1:4$; but for the mother's father's mother of a female zygote $1:C_x = (1:1) . (1:2) . (1:1) = 1:2$.

For an ancestor not in the X-trail, $1 : C_x = 1:\infty$.

Thus, the so-called "X-trail"—from a *son to the mother only*, but from a *daughter to both the father and mother*, traces the several lines in the ancestral pedigree down *one* of which an X-chromosome *must* descend.

NOTE: The X-trail and the Y-trail and all calculations herein based upon them apply only to those species in which the male is the heterozygous and the female the homozygous sex. In species in which the reverse is true the X- and the Y-trails exchange positions. The general rule is that the Y-trail follows the heterozygous sex; the X-trail from the homozygous individual to both parents, but from a heterozygous individual to the homozygous parent only.

6. R = range in number of chromosomes (i.e., in number of single members of chromosome-pairs) possible for the selected ancestor, taken as a type, to contribute to the 24 (i.e., 11 equivalent pairs and 1 XY or 1 XX pair) chromosomes of the F_1 zygote.

In a parent of the P_1 generation, $R = 12 \rightarrow 12$, i.e., there is no range in the number of chromosomes which the given parent may contribute to the F_1 zygote; this number is always 12. For all ancestors of the P_2 and all older generations, $R = 0 \rightarrow 11$, except (a) for those ancestors from whom an X- or a Y-chromosome *must* certainly descend (solid line X-trail or Y-trail on chart), in whom $R = 1 \rightarrow 12$; except also (b) for those ancestors from whom an X-chromosome *may*, by alternative chance, descend (dotted line X-trail in chart), in whom $R = 0 \rightarrow 12$.

In a general formula 11 would be replaced by n, and 12 by $n + 1$, with the value of n as in formula 2.

7. $1:C_t$ = the probability (barring mutation, crossing over and other special phenomena) that *one or the other* member of *each* of *all* of the *equivalent* chromosome-pairs characteristic of the species (i.e., an entire chromosome-series excluding the XX and XY pairs), from the given ancestor will enter the F_1 zygote.

In the case of ancestors not in the X-trail or the Y-trail, such an occurrence is the extreme limit of ancestral influence possible to a given ancestor, and its happening precludes any hereditary influence at all on the propositus, i.e., the F_1 zygote, on the part of any other ancestor of the same paternal or maternal half-pedigree, as the case may be, except the lineal ancestors and descendants of the given ancestor; consequently $1:C_t$ also measures the probability that the selected ancestor will contribute all of the representatives of the equivalent chromosome-pairs entering the F_1 zygote from the particular half-pedigree and generation.

$1:C_t = 1:(2^{(p-1)})^n$

n = value as in formula 2. In man n is taken as 11.

p = value as in formula 3.

NOTE: Formula 7 is in fact a special case of formula 17.

8. $1:C_{tx}$ or $1:C_{ty}$ = the probability (barring mutation, crossing over and other special phenomena) that *one or the other* member of *each* of the several equivalent chromosome-pairs characteristic of the species, and *also* one X- or one Y-chromosome, as the case may be, from the given ancestor will enter the F_1 zygote. (In man this total gametic number of chromosomes is taken as 12.)

Such an occurrence can happen only in reference to ancestors in the X- or Y-trails, and its happening precludes any hereditary influence at all on the propositus, i.e., the F_1 zygote, on the part of any other ancestor of the same paternal or maternal half-pedigree, except the lineal ancestors and descendants of the given ancestor.

$$1:C_{tx} = (1:C_t) \,.\, (1:C_x)$$

Values of $1:C_t$ and $1:C_x$ as in formulas 5 and 7.

$$1:C_{ty} = (1:C_t) \,.\, (1:C_y)$$

Values of $1:C_t$ and $1:C_y$ as in formulas 4 and 7.

9. $1:C_{-t}$ = the probability (barring mutation, crossing over and other special phenomena) that *no member* of *any* of the equivalent chromosome-pairs characteristic of the species (thus barring for the time being all consideration of the XY or XX chromosome-pairs) from the given ancestor will enter the F_1 zygote.

In such an occurrence the given ancestor and all of his lineal ancestors are *entirely eliminated* as contributors to the natural inheritance of the propositus, i.e., the F_1 zygote, *provided* that such given ancestor is not in the X-trail or Y-trail, and does not, due to inbreeding, appear elsewhere in the ancestral pedigree.

$$1:C_{-t} = 1:\left(\frac{2^{(p-1)}}{2^{(p-1)} - 1}\right)^n$$

n = value as in formula 2.

p = value as in formula 3.

$1:C_{-t}$ measures the probability that a selected ancestor will contribute none of the representatives of the equivalent chromosome-pairs entering the F_1 zygote from the particular half-pedigree.

Therefore, the probability that the selected ancestor will contribute all of the representatives of the equivalent chromosome-pairs equals the probability that all of the remaining members of the same ancestral generation of the same half-pedigree will be left out entirely as contributors of members of equivalent chromosome pairs to the F_1 zygote; whence the check for formula 7,

$$1:C_t = 1:(C_{-t}) \cdot (2^{(p-1)} - 1)^n$$

in which $2^{(p-1)} - 1$ is always the number of ancestors of the particular generation of the same half-pedigree who contribute nothing in case the one remaining member contributes all, and in which n equals the number of equivalent chromosome-pairs characteristic of the species.

Still more generally the probability that a given combination of members of the same ancestral generation of the same half-pedigree will contribute no representative of the equivalent chromosome-pairs to the F_1 zygote equals $1:(C_{-t}) \cdot m^n$ in which m is the number of members of the selected combination of ancestors of the same generation and the same half-pedigree which cannot exceed $2^{(p-1)} - 1$.

NOTE; It is apparent that the probability that a given ancestor will contribute *no* member of any equivalent chromosome pairs to the F_1 zygote (formula 9), is quite different from the probability that a given ancestor *will not contribute all* of the chromosome representative of the entire chromosomal series. The latter is the negative of formula 7, which negative is stated, $1:\dfrac{(2^{(p-1)})^n}{(2^{(p-1)})^n - 1}$

NOTE: Formula 9 is in fact a special case under formula 19.

10. $1:C_{-tx}$ or $1:C_{-ty}$ = the probability (barring mutation, crossing over and other special phenomena) that *no member* of *any* equivalent chromosome-pair, *nor* of the XX or XY chromosome-pair, from the given ancestor will enter the F_1 zygote, i.e., the probability (barring crossing over and other special phenomena, and the appearance of the given ancestor elsewhere in the pedigree) of the *complete elimination* of the hereditary influence of the given ancestor and all of his or her lineal ancestors, on the propositus, i.e., the F_1 zygote.

This formula is applicable *only* to those ancestors *in the X-trail or Y-trail.*

$1:C_{-tx} = (1:C_{-t}) \cdot (1:C_{-x})$

Values of $1:C_{-t}$ and $1:C_{-x}$ as in formula 9 above and 11 following.

$1:C_{-ty} = (1:C_{-t}) \cdot (1:C_{-y})$

Values of $1:C_{-t}$ and $1:C_{-y}$ as in formula 9 above and 11 following.

11. $1:C_{-x}$ = the probability that a given ancestor who is in the X-trail will *not* contribute an X-chromosome to the F_1 zygote.

$$1:C_{-x} = 1:\frac{C_x}{C_x - 1}$$

For an ancestor not in the X-trail, $1:C_{-x} = 1:1$.

Value of C_x as in formula 5.

12. $1:C_{-y}$ = the probability that a given ancestor who is in the Y-trail will *not* contribute a Y-chromosome to the F_1 zygote.

$$1:C_{-y} = 1:\frac{C_y}{C_y - 1}$$

For an ancestor not in the Y-trail, $1:C_{-y} = 1:1$.

Value of C_y as in formula 4.

13. $1:G_a$ = the probability that *one or the other* member of an allelomorphic pair of Mendelian genes (disregarding genes in the XX or XY sex-chromosomes), regardless of whether the selected member is dominant or recessive to its allelomorph, from the given ancestor will enter the F_1 zygote.

$1:G_a = 1:2^{(p-1)}$

$G_a = 2^{(p-1)}$ = the number of ancestors in a givèn half-generation.

$1:G_a$ = also the probability that the *entire chromosome*, (barring crossing over, non-disjunction, and other special phenomena), of which the particular gene is a part will enter the F_1 zygote.

p = value as in formula 3.

NOTE: This ratio may well be called the foundation formula of human heredity. As biological knowledge advances other subsidiary formulae may be deduced and synthesized with it.

14. $1:G_{-a}$ = the probability that *neither member* of a given allelomorphic pair of Mendelian genes (disregarding genes in the XX or XY sex-chromosomes) from the given ancestor will enter the F_1 zygote.

$$1:G_{-a} = 1:\frac{1}{1 - \frac{1}{2^{(p-1)}}}$$

p = value as in formula 3.

$1:G_{-a}$ = also the probability that *neither* member of the *entire chromosome-pair*, barring crossing over, non-disjunction and other special phenomena, of which the particular gene is a part, will enter the F_1 zygote.

15. $1:G_b$ = the probability that a *definitely selected paternally or maternally contributed* member of an allelomorphic pair of Mendelian genes (disregarding genes in the XX or XY sex-chromosomes),

regardless of whether the particular member is dominant or recessive to its allelomorph, from the given ancestor will enter the F_1 zygote.

$1:G_b = 1:2^p$

$1:G_b$ = also the probability that, barring crossing-over, non-disjunction and other special phenomena, the *entire chromosome*, of which the particular gene is a part will enter the F_1 zygote.

p = value as in formula 3.

16. $1:G_{\neg b}$ = the probability that a *definitely selected paternally or maternally contributed* member of a pair of Mendelian genes, (disregarding genes in the XX or XY sex-chromosomes), from the given ancestor will *not* enter the F_1 zygote.

$$1:G_{\neg b} = 1:\frac{1}{1-\frac{1}{2^p}}$$

p = value as in formula 3.

$1:G_{\neg b}$ = also the probability that, barring crossing over, non-disjunction and other special phenomena, the *entire chromosome* of which the particular gene is a part will *not* enter the F_1 zygote.

17. $1:C_a$ = the probability (barring mutation, crossing over and other special phenomena) that *one or the other* member from each of a *definitely selected combination* of equivalent chromosome-pairs (i.e., a definite combination among the chromosomes a to k, if 11 be the number of such equivalent pairs in man, thus disregarding the alternative XX and XY sex-pairs), from the given ancestor, regardless of the behavior of other chromosome-pairs, will enter the F_1 zygote.

$1:C_a = 1:(2^{(p-1)})^a$

p = value as in formula 3.

a = the number of such chromosomes in the definitely selected combination, (which in man cannot exceed 11).

$1:(2^{p-1})^a$ = also the probability that a *definite combination of one of the other allelomorphs* of non-linked genes, barring crossing over, non-disjunction and other special phenomena, carried by the given ancestor, will enter the F_1 zygote.

18. $1:C_b$ = the probability (barring mutation, crossing over, and other special phenomena) that a *definitely selected paternally or maternally* contributed member from *each* of a *definitely selected combination* of equivalent chromosome-pairs (i.e., a definite combination among chromosomes a to k, if 11 be the number

of such equivalent pairs in man, thus disregarding the alternative XX and XY sex-pairs) from the given ancestor, regardless of the behavior of other chromosome-pairs, will enter the F_1 zygote.

$1:C_b = 1:(2^p)^b$

p = value as in formula 3.

b = number of definitely selected paternally or maternally descended chromosomes in the definite combination passed on by the given ancestor.

$1:(2^p)^b$ = also the probability that a definite combination of definitely selected paternally or maternally descended *non-linked* genes, barring crossing over, non-disjunction and other special phenomena, carried by the given ancestor will enter the F_1 zygote.

19. $1:C_{-c}$ = the probability that *neither* member of any pair of a *definitely selected* combination of equivalent chromosome-pairs (i.e., a definite combination among chromosome-pairs a to k, if 11 be the correct number in man, thus disregarding the alternative XX and XY sex-pairs) from the given ancestor, regardless of the behavior of other chromosome-pairs, will enter the F_1 zygote.

$$1:C_{-c} = 1:\frac{1}{\left(1 - \frac{1}{2^{(p-1)}}\right)^c}$$

c = number of chromosomes in the definitely selected combination barred from the F_1 zygote. In man it must not be greater than 11.

p = value as in formula 3.

$1:C_{-c}$ = also the probability that *neither* allelomorph of a *definitely selected combination* of *non-linked* Mendelian genes, barring crossing over, non-disjunction and other special phenomena, carried by the given ancestor will enter the F_1 zygote.

20. $1:C_d$ = the probability (barring mutation, crossing-over and other special phenomena) that *one or the other member* of a *definitely selected combination* of equivalent chromosome-pairs (definite combinations among the chromosome-pairs a to k, if 11 be the correct number of equivalent chromosome-pairs in man, thus disregarding the alternative XX and XY sex-pair), and *no more* chromosomes from the given ancestor will enter the F_1 zygote.

$$1 : C_d = 1 : \frac{\left(2^{(p-1)}\right)^a}{\left(1 - \frac{1}{2^{(p-1)}}\right)^c}$$

a = value as in formula 17.
n = value as in formula 2.
p = value as in formula 3.
c = value as in formula 19.

NOTE: This formula is the product of formula 17 times formula 19, in which always the number of chromosomes entering plus the number barred from the F₁ zygote = n, i.e., $a + c = n$.

21. $1 : C_e$ = the probability (barring mutation, crossing over and other special phenomena) that a *definitely selected paternally or maternally* contributed member from each of a *definitely selected combination* of equivalent chromosome-pairs (i.e., a definite combination among chromosome-pairs a to k, if 11 be the number of such equivalent pairs in man, thus disregarding the alternative XX and XY sex-pairs), and *no more* chromosomes, from the given ancestor, will enter the F_1 zygote.

$$1 : C_e = 1 : \frac{\left(2^{p}\right)^b}{\left(1 - \frac{1}{2^{(p-1)}}\right)^c}$$

b = value as in formula 18.
n = value as in formula 2.
p = value as in formula 3.
c = value as in formula 19.

NOTE: This formula is the product of formula 18 times formula 19, in which always the number of chromosomes entering plus the number barred from the F_1 zygote = n, i.e., $a + c = n$.

22. $1 : N_a$ = the probability (barring mutation, crossing over, and other special phenomena) that a *definite number* (but no more) of chromosomes from the given ancestor, regardless of their individuality, and of their paternal or maternal descent, will enter the F_1 zygote.

$$1 : N_a = 1 : \frac{\dfrac{\left(2^{(p-1)}\right)^d}{\left(1 - \frac{1}{2^{(p-1)}}\right)^c}}{\dfrac{n \cdot (n-1) \cdot (n-2) \cdot (n-3) \ldots . (n-d+1)}{1 \cdot 2 \cdot 3 \ldots . d}}$$

d = the ***definite number*** of chromosomes (regardless of combinations) passed on to the F_1 zygote.

n = value as in formula 2.

p = value as in formula 3.

$c = n - d$.

23. $1:N_b$ = the probability (barring mutation, crossing over and other special phenomena) that a ***definite number*** (but no more) of chromosomes from the given ancestor, regardless of their individuality, but ***specified in total numbers as to the maternity and paternity*** of their descent, will enter the F_1 zygote.

$$1:N_b = 1:\frac{\dfrac{(2^{(p-1)})^d\cdot 2^d}{\left(1-\dfrac{1}{(2^{(p-1)})}\right)^c}}{\dfrac{n\cdot(n-1)\cdot(n-2)\cdot\ldots\cdot(n-d+1)}{1\cdot 2\cdot 3\ldots d}\times\dfrac{d\cdot(d-1)\cdot(d-2)\ldots(d-s+1)}{1\cdot 2\cdot 3\ldots s}}$$

d = value as in formula 22.

n = value as in formula 2.

p = value as in formula 3.

$c = n - d$.

s = the number of chromosomes originating in the selected parent of the given ancestor, that will enter the F_1 zygote.

$d - s$ = the number of chromosomes originating in the other parent of the given ancestor, that will enter the F_1 zygote.

24. In case of inbreeding or consanguineous matings, the measure of ancestral influence (barring mutation, crossing over, and other special phenomena) is determined as follows:

(a) A_m = the average number of chromosomes which a given ancestor, taken as a type, who appears more than once in the direct ancestry, contributes to the propositus.

A_m = the sum of the several values of A for each of the several appearances of the particular ancestor in the pedigree.

(b) R_m = the range in possible number of chromosomes which a given ancestor, taken as a type, who appears more than once in the direct ancestry, contributes to the propositus.

$R_m = r' \rightarrow r''$ (i.e., from r' to r'').

r' = the ***highest*** of the several ***minima*** in the several values of R for the given ancestor in his or her several places in the direct ancestry.

r'' = the *highest* of the several *maxima* in the several values of R for the given ancestor in his or her several places in the direct ancestry.

(c) $1:C_{tmx}$ = the probability that a given ancestor, taken as a type, who appears more than once in the direct ancestry, will contribute from *one or more* of the several such appearances *all* of the chromosomes supplied by the particular half-pedigree to the propositus.

$$1:C_{tmx} = 1 : \frac{\left(\frac{h}{g}\right)^n}{(1:C_x)\cdot(1:C_y)}$$

n = value as in formula 2.
h = the total number of direct ancestors in the oldest half-generation in which the particular inbred ancestor appears ($=2^{(p-1)}$).
p = value as in formula 3.
g = the number of parts of the particular half-pedigree covered by the given ancestor, in terms of h units.
C_x = value as in formula 4.
C_y = value as in formula 5.

(d) $1:C_{-tmx}$ = the probability that a given ancestor, taken as a type, who appears more than once in the direct ancestry will contribute *no* chromosomes to the propositus.

$$1:C_{-tmx} = 1 : \left(\frac{\frac{h}{g}}{\frac{h}{g} - 1}\right)^n \cdot (C_{-xa}\cdot C_{-xb}\cdots)\cdot(C_{-y})$$

n = value as in formula 2.
h = value as in formula 24(c).
g = value as in formula 24(c).
C_{-x} = value as in formula 11. (a, b, and so on, following the x in the formula, refers to C_{-x} in the first, second and other appearances of the propositus in the X-trail).
C_{-y} = value as in formula 12.

It is obvious that 24(c) or 24(d), if in the given case the *last* two parentheses be omitted, measures the given probability in reference to all of the chromosomes except the X and Y sex-members. Also if n represent a definite combination of n

specific chromosomes, regardless of the maternity or paternity of their descent and regardless of the behavior of other chromosomes, the formula without the last two parentheses is true for the specific chromosomal combination selected. Also these same formulae without the *first* parenthesis measure the given probability for the X and Y sex-chromosomes.

(e) The probability that the propositus will be a zygotic chromosomal replica of a given ancestor who appears once or more in both the paternal and maternal half-pedigrees is equal to the product of the probability-quotient that the particular inbred ancestor will supply all of the chromosomes contributed to the F_1 zygote by the paternal half-pedigree (formula 24(c)) *times* the same probability-quotient in reference to the maternal half-pedigree *times* $1:2^{(n+1)}$.

n = value as in formula 2.

It is obvious that in the case of species with XY-type of male and XX-type of female a male replica can occur only if the repeating ancestor is a male and appears at least in both the X- and Y-trails; the female replica only if the repeating ancestor is a female and appears at least in the X-trail in both the paternal and maternal sides of the house. The formula, however, takes all of these chances into consideration.

A zygotic chromosomal replica in relation to its repeated ancestor is biologically the equivalent of one identical twin to its mate, although in the case of chromosomal replicas the natural traits have been segregated, distributed among several direct ancestors and later reassembled in the replica, while in the case of identical twins the duplication is immediate from the zygote.

25. S = the minimum number of ancestors in a given ancestral generation among whom will be found all of the possible contributors of the sex-chromosomes (i.e., XX or XY) to the F_1 zygote.

p = the number of the given ancestral generation: e.g., for parents, $p = 1$; for grandparents, $p = 2$; for great-grandparents, $p = 3$; and so on as in formula 3.

In the human female in which the sex-chromosome formula is X + X, S = the $(p + 3)$th member of the Fibonacci series. The maternal half-pedigree provides one X-chromosome in which half-pedigree S = Fibonacci serial term $p + 2$. In the paternal half-pedigree which also contributes one X-chromosome, S = Fibonacci serial term $p + 1$.

In the human male the maternal half-pedigree contributes an X-chromosome, the paternal half-pedigree a Y-chromosome. In this sex for both half-pedigrees united, $S =$ (Fibonacci serial term $p + 2$) $+ 1$. In the maternal half pedigree $S =$ Fibonacci serial term $p + 2$, in the paternal half-pedigree $S = 1$.

Fibonacci series = 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, ..., in which each term is the sum of the two preceding terms.

SUMMARY

1. The present problem of measuring ancestral influence by tracing chromosomes or gene-radicals is demonstrated to be a special case under the following general situation:

Given:

(a) A single biological unit factor (a gene) in a known situation (in a P_1 ancestor) and the quotient measuring the probability of its reacting in a definite manner or reaching a definite new situation at the end of a single move or transformation (in the F_1 zygote).

(b) The demonstrated synchronization or sequence of action of two or more such biological unit factors (several genes through several generations).

Solution:

By applying the correct principles of combination and chance, the probability that a given complex situation will result from a given set of constituent conditions at the end of a given number of definite kinds of transformations is mathematically formulated.

In the case of measuring ancestral influence by tracing chromosomal descent, the situations and transformations concern definite chromosome-complexes in the zygote and their breaking-up, their recombination and their transmission to offspring in accordance with the principles demonstrated by genetics and cytology.

2. The formulae of bisexual heredity here set forth are foundational. They are stated in general terms whose validity depends upon their presenting correct mathematical pictures of the chromosomal processes which work out in gametogenesis and fertilization.

3. When knowledge of a special case or type of chromosomal behavior is worked out, the mathematical formula which describes the particular case may be joined to the foundational formulae here given, and thus the the general mathematical formula of bisexual heredity may be gradually approximated.

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LEGEND FOR ♂ CHROMOSOME-ORIGIN CHART (PLATE 1)

NOTES: The calculations on this chart (a) are based upon the theory that the zygotic chromosome-formula for the human male is (11 + 1X) + (11 + 1Y), and (b) do not account for mutation, crossing over, special schemes of selection or other special phenomena.

The formulae upon which these calculations are based are general and foundational, and apply to any bisexual species for which the chromosome-number is given. They may be corrected to account for special phenomena by the articulation of formulae for particular processes when the latter are mathematically generalized.

A = average number of chromosomes passed on by the selected ancestor to the F_1 zygote. (Formula 1.)

ΣA for all of the ancestors of a given generation = 24.

R = range within the haploid number of chromosomes (i.e., number of single members of chromosome pairs) possible for the selected ancestor to contribute to the 24 (i.e., 11 equivalent pairs and the XY sex-pair) chromosomes of the F_1 zygote. Barring crossing over each actual contribution is a whole number. (Formula 6.)

C_t = the chance that one or the other member of each equivalent chromosome pair, i.e., an entire haploid series, excluding a member of the XY sex-pair, in a given ancestor will enter the F_1 zygote. (Formula 7.)

C_{-t} = the chance that no member of any equivalent chromosome pair in a given ancestor will enter the F_1 zygote. (Formula 9.)

C_x = the chance that the selected ancestor carried the single X-chromosome that will enter the F_1 ♂ zygote. (Formula 5.)

Also = the average number of X-chromosomes contributed by the selected ancestor to the F_1 zygote.

C_y = the chance that the selected ancestor carried the single Y-chromosome that will enter the F_1 ♂ zygote. (Formula 4.)

Also = the average number of Y-chromosomes contributed by the selected ancestor to the F_1 zygote.

C_{tx} or C_{ty} = the chance that one or the other member of each equivalent chromosome pair and also one X- or Y-chromosome in the selected ancestor, will enter the F_1 zygote, i.e., that the selected ancestor represents all of the ancestral influence of her or his generation in her or his half-pedigree. (Formula 8.)

C_{-tx} or C_{-ty} = the chance that no member of any equivalent chromosome pair nor of the XX or XY sex-pair in the selected ancestor will enter the F_1 zygote, i.e., the chance of complete elimination of the ancestral influence of the selected ancestor. (Formula 10.)

For the F_1 male zygote

Certain selected formula-values for given ancestors in the P_4 generation

(P_4 1)

$A = 1.375 + 1Y$

$R = 1 \rightarrow 12$

$C_{ty} = 1:8,589,934,592$

$C_{-ty} = 0:\infty$

$C_y = 1:1$

(P_4 2, 3, 4, 5, 6, 7, 8, 9, 10, and 13)
$A = 1.375$
$R = 0 \rightarrow 11$
$C_t = 1:8,589,934,592$
$C_{-t} = 1:4.3442+$

(P_4 11, 12, and 14)
$A = 1.375 + 0.25X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:34,359,738,368$
$C_{-tx} = 1:5.7922+$
$C_x = 1:4$

(P_4 15 and 16)
$A = 1.375 + 0.125X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:68,719,476,736$
$C_{-tx} = 1:4.9648+$
$C_x = 1:8$

For the F_1 male zygote

Certain selected formula-values for given ancestors in the P_5 generation

(P_5 1)
$A = 0.6875 + 1Y$
$R = 1 \rightarrow 12$
$C_{ty} = 1:17,592,186,044,416$
$C_{-ty} = 0:\propto$
$C_y = 1:1$

(P_5 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 25, 26, and 29)
$A = 0.6875$
$R = 0 \rightarrow 11$
$C_t = 1:17,592,186,044,416$
$C_{-t} = 1:2.0338+$

(P_5 22)
$A = 0.6875 + 0.25X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:70,368,744,177,664$
$C_{-tx} = 1:2.7117+$
$C_x = 1:4$

(P_5 23, 24, 27, 28, and 30)
$A = 0.6875 + 0.125X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:140,737,488,355,328$
$C_{-tx} = 1:2.3243+$
$C_x = 1:8$

(P_5 31 and 32)
$A = 0.6875 + 0.0625X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:281,474,976,710,656$
$C_{-tx} = 1:2.1693$
$C_x = 1:16$

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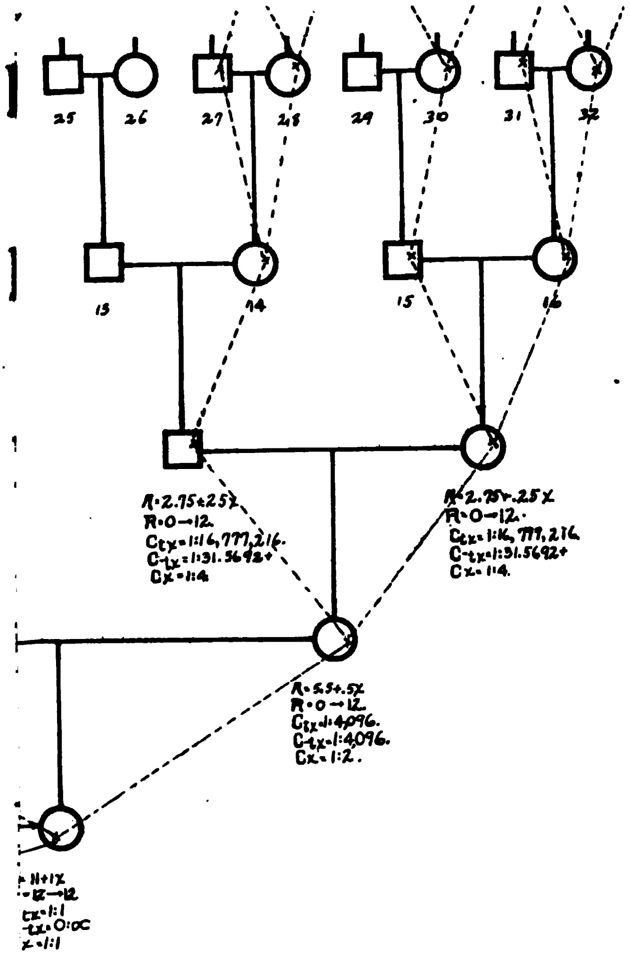
ote, actually passed on by all of F1's maternal ancestors of



F.

LEGEND FOR ♀ CHROMOSOME-ORIGIN CHART (PLATE 2)

NOTE: These calculations (a) are based upon the theory that the zygotic chromosome-formula for the human female is $(11 + 1X) + (11 + 1X)$, and (b) do not account for mutation, crossing over, special schemes of selection or other special phenomena.

A = average number of chromosomes passed on by the selected ancestor to the F_1 zygote. (Formula 1.)

ΣA for all of the ancestors of a given generation = 24.

R = range within the haploid number of chromosomes (i.e., the number of single members of chromosome pairs) possible for the selected ancestor to contribute to the 24 (i.e., 11 equivalent pairs and an XX pair) chromosomes of the F_1 zygote. Barring crossing over each actual contribution is a whole number. (Formula 6.)

C_t = the chance that one or the other member of each equivalent chromosome-pair (i.e., an entire haploid series excluding the X-chromosome) in a given ancestor will enter the F_1 zygote. (Formula 7.)

C_{-t} = the chance that no member of any equivalent chromosome pair, excluding the XY sex-pair, in a given ancestor will enter the F_1 zygote. (Formula 9.)

C_x = the chance that the selected ancestor carried one of the X-chromosomes that will enter the F_1 ♀ zygote. (Formula 5.)

Also = the average number of X-chromosomes contributed by the selected ancestor to the F_1 zygote.

C_{tx} = the chance that one or the other member of each equivalent chromosome-pair and also one X-chromosome in the selected ancestor will enter the F_1 zygote, i.e., that the selected ancestor represents all of the ancestral influence in her or his generation in her or his half-pedigree. (Formula 8.)

C_{-tx} = the chance that no member of any equivalent chromosome-pair nor of the XX or XY sex-pair in the selected ancestor will enter the F_1 zygote, i.e., the chance of complete elimination of the ancestral influence of the selected ancestor. (Formula 10.)

For the F_1 female zygote

Certain selected formula-values for given ancestors in the P_4 generation

(P_4 1, 2, 3, 4, 5, 9, 10, and 13)
$A = 1.375$
$R = 0 \rightarrow 11$
$C_t = 1:8{,}589{,}934{,}592$
$C_{-t} = 1:4.3442+$

(P_4 6)
$A = 1.375 + 0.5X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:17{,}179{,}869{,}184$
$C_{-tx} = 1:8.6884+$
$C_x = 1:2$

(P_4 7, 8, 11, 12, and 14)
$A = 1.375 + 0.25X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:34{,}359{,}738{,}368$
$C_{-tx} = 1:5.7922+$
$C_x = 1:4$

(P_4 15 and 16)
$A = 1.375 + 0.125X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:68{,}719{,}476{,}736$
$C_{-tx} = 1:4.9648+$
$C_x = 1:8$

For the F_1 female zygote

Certain selected formula-values for given ancestors in the P_5 generation

(P_5 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 17, 18, 19, 20, 21, 25, 26, and 29)
$A = 0.6875$
$R = 0 \rightarrow 11$
$C_t = 1:17{,}592{,}186{,}044{,}416$
$C_{-t} = 1:2.0338+$
(P_5 11, 12, 14, and 22)
$A = 0.6875 + 0.25X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:70{,}368{,}744{,}177{,}664$
$C_{-tx} = 1:2.7117+$
$C_x = 1:4$
(P_5 15, 16, 23, 24, 27, 28, and 30)
$A = 0.6875 + 0.125X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:140{,}737{,}488{,}355{,}328$
$C_{-tx} = 1:2.3243+$
$C_x = 1:8$
(P_5 31 and 32)
$A = 0.6875 + 0.0625X$
$R = 0 \rightarrow 12$
$C_{tx} = 1:281{,}474{,}976{,}710{,}656$
$C_{-tx} = 1:2.1693+$
$C_x = 1:16$

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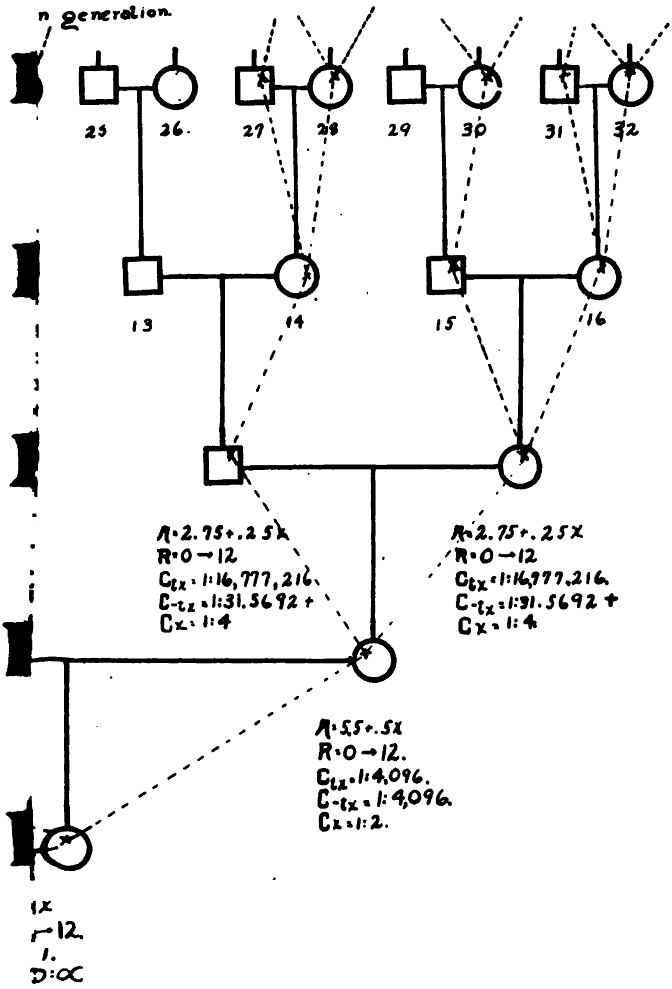
hing the F_1 zygote, actually passed on by all of F_1's maternal

n generation.



GENETIC AND CYTOLOGICAL EXAMINATION OF THE PHENOMENA OF PRIMARY NON-DISJUNCTION IN *DROSOPHILA MELANOGASTER*

SHELLEY R. SAFIR

Columbia University, New York City

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INTRODUCTION

The phenomena of non-disjunction, including the production of "exceptions" to sex-linked inheritance, take their origin in the failure of members of the pair of sex-chromosomes to segregate into separate gametes (Bridges 1913, 1914, and especially 1916). In individuals of the normal chromosomal constitution, such as XX females, non-disjunction was found to be of very rare occurrence; only about one egg in 1700 was found to be of the XX type or conversely of the no-X type. If such an XX egg produced by primary non-disjunction in a normal female is fertilized by a normal Y sperm then a matroclinous daughter of the constitution XXY is produced. The existence of XXY females has been demonstrated by Bridges both by direct cytological examination and by conclusive genetic tests. Matroclinous XXY females produce further exceptions, which are called secondary, to the extent of about 4.3 percent.

The cause of this production is the fact that the presence of the extra Y chromosome with its characteristic synaptic behavior forces both X's to enter the same egg in a certain percent of reductions. In his latest article on non-disjunction (1916) BRIDGES has reported very thorough genetic and cytological tests of the various phenomena of secondary non-disjunction, and his results furnish convincing proof of the chromosome theory of heredity.

In addition to the production of XXY females, primary non-disjunction was found to have other interesting results. The fertilization of the no-X egg by an X sperm was found by BRIDGES to give rise to a patroclinous son of the chromosome type XO. Such XO males proved to be entirely normal in somatic appearance both as to sex-linked characters (though "exceptions" to sex-linked inheritance) and as to sexual characters, but such XO males produced by primary non-disjunction were found to be absolutely sterile.

It was foreseen that adequate study of primary non-disjunction would be very difficult because of the fact that the cause or causes of primary non-disjunction were purely conjectural, and one must therefore depend upon the chance occurrence of primary non-disjunction for the working material.

At the suggestion of Prof. T. H. MORGAN and Dr. C. B. BRIDGES I undertook the following problems in connection with primary non-disjunction:

1. Large-scale tests of the frequency of occurrence of primary non-disjunction.
2. A study of the frequencies of primary non-disjunction in diverse stocks.
3. More precise tests of the degree and generality of the sterility of the patroclinous XO males produced by primary non-disjunction.
4. The cause or causes of such sterility.
5. Cytological demonstration of the constitution of the sterile patroclinous males produced by primary non-disjunction.

THE FREQUENCY OF PRIMARY NON-DISJUNCTION

In an experiment to test the constitution of patroclinous sons in secondary non-disjunction BRIDGES (1916) found 12 primary exceptions in a total of 20,484 flies. Various other experiments gave further information on the frequency of primary non-disjunction which seemed to be in the neighborhood of one in 2000 offspring.

For my more extensive tests of the frequency of primary exceptions, begun in the summer of 1914, flies homozygous for the two sex-linked recessive mutants, eosin eye color and miniature wings, were out-crossed to wild males. Normally when this cross is made all the sons, deriving their single X chromosome from their mother, show the characters,—in this case eosin and miniature,—whose genes are carried by the X

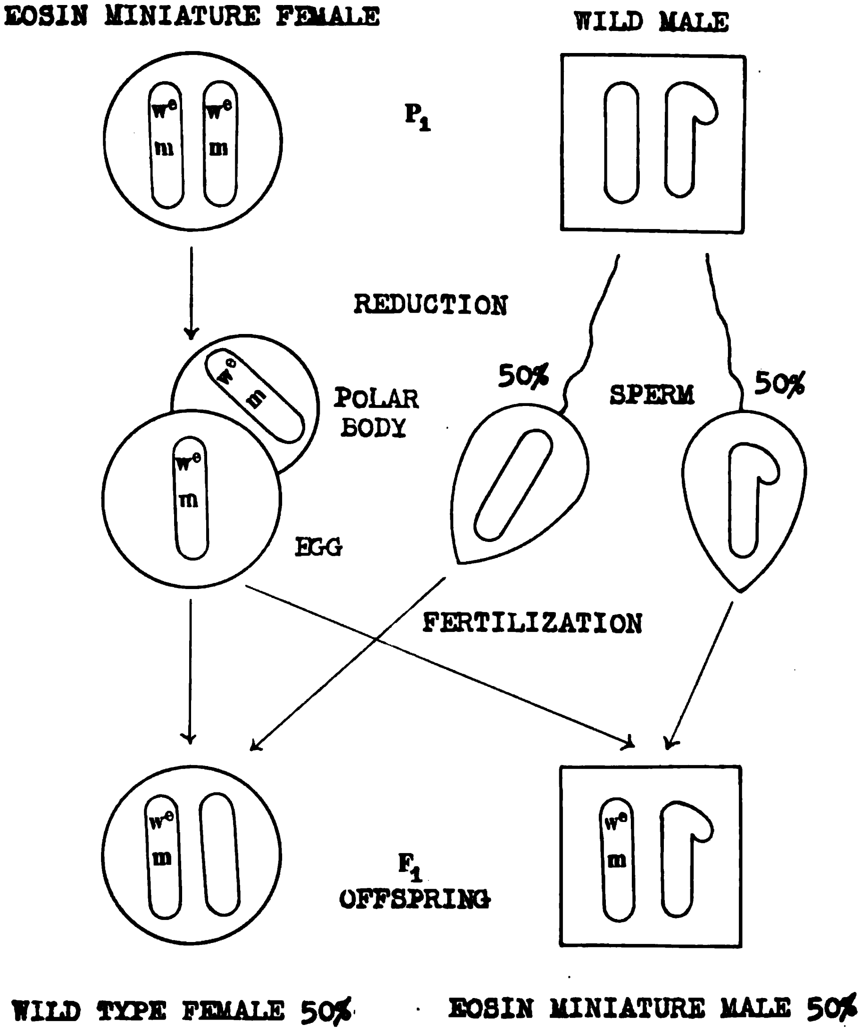


FIGURE 1.—Diagram illustrating normal criss-cross inheritance characteristic of sex-linkage. The genes for the two sex-linked mutations, eosin and miniature, are carried by the X chromosome (straight), the Y chromosome (J-shaped) is neutral with respect to these mutant characters.

chromosome of the mother. The daughters, however, fail to show these recessive sex-linked characters because the normal dominant allelomorphs of eosin and minature are brought in by the X chromosome from the father. Figure 1 shows a diagram of the normal course of inheritance in the above cross.

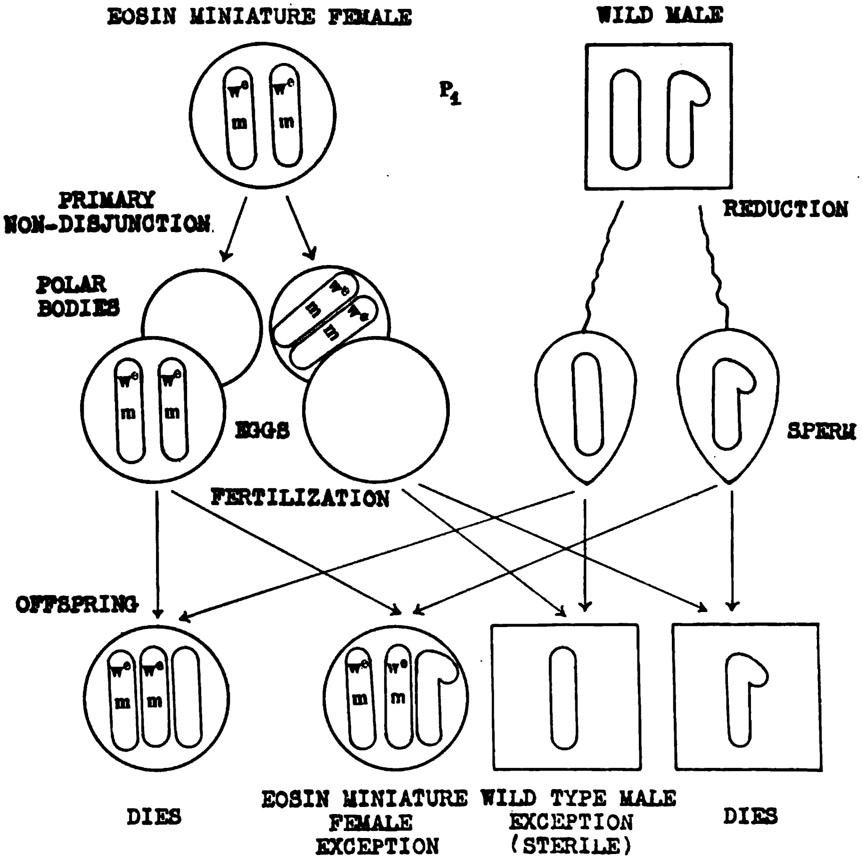


FIGURE 2.—Diagram to show primary non-disjunction of the X chromosomes in an eosin miniature female and the exceptions produced when the resulting XX and zero eggs are fertilized by X and Y sperm of a wild male.

In addition to these regular eosin miniature males and wild-type females two other classes of offspring, eosin miniature females and wild-type males, appeared in very small numbers. The appearance of these exceptions is due to the occurrence of primary non-disjunction. In a very small percent of the eggs, during the reduction division, the two X chromosomes fail to disjoin quickly enough and are both caught either in the egg or in the polar body, so that two abnormal types of eggs are produced. One type has two X chromosomes and correspondingly the other type has no X. Such eggs when fertilized by the X and Y sperm of a wild male give four abnormal types of zygotes XXX, XO, XXY, and YO. BRIDGES showed that both the YO and XXX zygotes are not viable, and hence fail to give any actual class among the progeny. Of the two remaining classes, the XXY becomes an exceptional matroclinous eosin-eyed, miniature-winged female, since both of her eosin miniature X chromosomes have come from the mother. The XO individual is a male since it has only one X, and is patroclinous, since this single X comes from his wild-type father.

These exceptions, produced by an XX female, are known as primary exceptions and the process which is responsible for their production is named primary non-disjunction. Figure 2 gives a diagram of the method of production of such exceptional gametes and zygotes.

Since in the experiments of BRIDGES the frequency of the occurrence of exceptions due to primary non-disjunction had been found to be in the neighborhood of one-twentieth of one percent, it was apparent that very large numbers would be required in order to secure data at all adequate. At first a long series of matings were started in which 245 cultures were raised from matings of single virgin eosin miniature females with one (or two) wild males.

TESTS OF THE NATURE OF THE EXCEPTIONS OF TABLE 1

An examination of the different cultures of table 1 makes it apparent that the results are in general very uniform. The great majority of the 245 cultures, namely 168, contained no exceptions at all, which is in accordance with a low frequency of production of primary exceptions. About a quarter of the cultures (namely 67) contained one exception each. In these 67 cultures the percentage of exceptions is so low that in all, except a few of the smallest, this fact alone is sufficient to prove that the exceptions in question were descended from XX mothers and were accordingly primary. Only seven cultures produced two exceptions each, and here again the percentage of exceptions (about one percent) is so low as to make it improbable that they are the result of secondary non-disjunction, which averages 4.3 percent of exceptions. Two cultures produced three exceptions each and one culture produced five exceptions. The culture which produced five exceptions (No. 261) is distinguished also by the fact that three of these exceptions were females. Both the

TABLE 1

Offspring given by eosin miniature females when out-crossed to wild males. Exceptions that were themselves tested by breeding are marked with an asterisk (*). Those cultures which were tested by tests of about 8 regular daughters are marked with a dagger (†).

No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions	
	+ ♀	$w^e m$ ♂	$w^e m$ ♀	+ ♂		+ ♀	$w^e m$ ♂	$w^e m$ ♀	+ ♂		+ ♀	$w^e m$ ♂	$w^e m$ ♀	+ ♂
1	64	56	—	—	53	62	41	—	—	*†106	102	95	—	1
2	82	79	—	—	56	45	62	—	—	107	101	97	—	—
3	60	67	—	—	57	68	60	—	—	108	103	97	—	—
*5	86	90	—	1	60	94	60	—	—	†109	76	70	—	1
6	91	83	—	—	*62	59	55	1	—	110	124	128	—	—
7	61	42	—	—	63	52	48	—	—	111	119	129	1	—
*†8	65	47	—	1	†65	75	56	—	1	112	101	89	—	—
*†9	84	83	1	—	*†67	85	77	—	1	113	121	138	—	—
10	103	108	—	—	69	84	73	—	1	114	103	96	—	—
12	82	66	—	—	70	108	97	—	—	115	98	107	—	—
*13	126	100	—	1	71	141	101	—	—	116	131	101	1	—
*†14	48	34	—	1	73	134	123	—	—	†117	144	101	—	1
17	93	79	—	—	74	103	121	—	1	118	105	98	—	—
18	45	48	—	—	75	84	75	—	—	120	110	85	—	—
*†19	72	85	—	2	76	88	76	—	—	121	119	92	—	1
21	48	27	—	—	77	98	101	—	—	122	131	111	—	—
23	76	75	—	—	78	118	130	—	—	123	128	116	—	—
*25	72	44	1	—	79	59	65	—	—	125	95	96	—	—
*†26	115	89	—	1	†81	95	87	—	1	†126	102	92	—	1
*27	84	69	1	—	83	115	112	—	—	128	138	129	—	—
29	27	22	—	—	84	102	100	1	—	129	163	147	—	—
*30	130	101	—	1	85	128	109	—	1	130	93	99	—	—
31	120	115	—	—	86	88	91	—	—	133	101	107	—	—
32	136	104	—	—	87	111	100	—	—	134	126	119	—	—
34	120	104	—	1	88	102	97	—	—	135	113	72	—	—
35	46	38	—	—	89	145	138	—	—	136	111	121	—	—
36	51	48	—	—	90	107	91	—	—	*†137	80	63	—	1
37	79	69	—	—	91	100	103	—	—	140	112	108	—	—
38	67	69	—	—	92	117	109	—	—	142	104	121	—	—
39	89	87	—	—	†93	112	101	—	1	143	118	70	—	—
40	51	52	—	—	95	119	132	—	1	*†144	100	89	—	1
†41	51	43	—	1	96	112	122	—	—	145	105	97	—	—
43	48	47	—	—	97	125	110	—	—	146	108	69	—	—
44	48	46	—	—	98	177	165	—	—	148	119	114	—	—
45	121	117	—	1	99	113	116	—	—	151	101	101	—	1
47	69	63	—	—	100	120	108	—	—	152	105	97	—	—
48	82	62	—	—	*102	114	126	—	2	153	105	92	—	—
*49	75	75	1	—	103	103	91	—	—	*154	85	98	—	2
50	138	117	—	—	104	99	90	—	—	*†155	142	109	—	1
51	156	129	—	—	105	107	131	—	—	156	95	85	—	—

TABLE 1 (continued)

No.	Regular offspring		Exceptions	
	+ ♀	$w^e m$ ♂	$w^e m$ ♀	+ ♂
158	99	103	1	—
159	108	91	—	1
160	121	102	—	—
161	113	94	—	—
*163	110	85	—	1
164	117	90	—	—
165	120	134	—	—
168	101	77	—	—
169	98	98	—	—
170	104	71	—	1
173	167	98	—	—
174	102	91	—	—
*175	111	86	—	2
176	94	88	—	—
177	124	85	—	1
*†178	79	67	—	1
179	86	79	—	—
180	114	98	—	1
181	98	110	—	—
182	103	112	—	1
183	87	67	—	—
186	114	58	—	1
187	132	90	—	—
188	122	86	—	—
189	129	85	—	—
192	133	98	—	—
193	103	86	—	—
*†194	106	99	—	3
195	114	110	—	—
196	86	70	—	1
198	95	92	—	—
199	106	97	—	—
200	139	108	—	—
201	116	121	—	—
202	102	107	—	—
203	106	76	—	—
205	131	118	—	1
206	95	93	1	—
207	149	137	—	—
208	152	138	—	—
209	92	76	—	1
210	122	104	—	—
212	145	126	—	—
213	109	108	—	—
214	135	135	—	—
*215	118	125	1	—
216	83	69	—	1
218	81	75	—	—
219	128	113	—	—
220	103	97	—	—
221	75	85	—	1
222	167	113	—	—
223	80	84	—	—
225	90	89	—	1
226	106	91	—	—
227	132	115	—	—
*†228	162	129	1	2
229	121	111	—	—
230	82	52	—	—
231	99	96	—	—
232	117	105	—	—
234	96	128	—	—
235	140	114	—	—
237	132	120	—	—
238	129	120	—	1
239	105	102	—	—
240	139	126	—	—
241	123	113	—	1
243	90	68	—	—
244	92	111	—	—
*†245	118	85	1	—
*†246	91	84	—	1
247	113	116	—	1
248	92	74	—	—
249	118	121	—	—
250	109	62	—	—
251	105	82	—	—
252	90	101	—	—
*253	151	125	—	1
254	97	77	—	—
256	100	95	—	1
257	123	86	—	—
259	102	102	—	—
260	112	103	—	—
*†261	126	98	3	2
262	115	93	—	—
263	79	97	—	—
*264	77	62	1	—
265	100	85	—	1
266	107	83	—	—
267	113	87	—	—
268	107	112	—	—
269	61	60	—	—
*271	79	73	—	2
272	99	101	—	—
273	104	98	—	—
274	75	72	—	—
275	70	74	—	—
*276	105	86	—	1
277	100	82	—	—
279	98	71	—	—
280	107	103	—	—
*281	104	101	—	2
282	64	66	—	—
283	64	67	—	1
*284	118	94	1	1
285	108	101	—	—
286	106	105	—	—
287	101	95	—	—
288	93	94	—	—
289	106	101	1	—
290	104	75	—	—
293	123	110	—	—
294	121	102	—	—
295	108	100	1	—
296	101	108	—	—
298	79	62	—	1
299	101	94	—	—
300	61	52	—	1
301	101	98	—	—
303	57	39	—	—
*304	101	94	—	1
305	100	86	—	—
306	77	60	—	—
307	102	107	—	—
310	42	22	—	—
Total*	25,004	22,454	17	70

*Exclusive of 261.

large number of exceptions and the equality of sexes mark this culture as the product of secondary non-disjunction. Further specific tests proved this to be true (see tables 2 and 9). In the case of the two cultures (194 and 228) that produced three exceptions each, the percentages of exceptions were still so low (1.5 and 1.0 percent, respectively), that they were probably due to primary non-disjunction, judging solely from that fact. However, in these cases certain other tests proved the mothers to have been XX in constitution and the exceptions primary (see tables 1 and 3).

The difference between the percentage of exceptions characteristic of primary and of secondary non-disjunction (0.05 percent versus 4.3 percent, according to the experiments of BRIDGES) was recognized as insufficient for a conclusive test of the constitution of the eosin miniature females. This source of confusion was foreseen and certain other tests were applied. BRIDGES had found that every male which from the pedigrees should have been a primary exception was absolutely sterile while the XY exceptional males produced by secondary non-disjunction behaved precisely like normal males and were correspondingly fertile. The number of primary exceptions that BRIDGES tested (15) while not sufficient to prove that all XO males are sterile, yet offered a test that could be applied tentatively to males suspected of being XO in constitution. The cultures in table 1 in which the sterility test was applied to exceptional males are marked with an asterisk. It was expected that the two males of culture 261 would prove fertile as in fact they did (table 2), proving in a third manner that that culture was an example of secondary non-disjunction. In all the other tests, however, the exceptional males proved absolutely sterile. The validity of this sterility test has since been completely justified (see page 000) so that we may regard the 34 cultures in which the exceptional males were sterile as being of proved XX descent.

TABLE 2

Offspring given by the two exceptional sons from culture 261 when tested by wild females

No.	Wild-type ♀	Wild-type ♂
261a	112	101
261b	127	119

The third test applied is the most conclusive of all since it depends upon the fact, established by BRIDGES, that half the regular daughters of an XXY female are themselves XXY and produce further secondary exceptions of the usual frequency (4.3 ±). Since the presence of the extra Y makes no somatic difference in the fly, enough such daughters must be tested to be sure of including at least one such XXY daughter. According to the laws of probability, where two classes occur in equal frequency, selection at random of one individual gives an even chance of securing the desired type, selection of two individuals gives a 3:1 chance of success and so on up. With 8 selections the chance of failure to include an XXY daughter would be one in 256 or practically negligible.

There were two other considerations that made tests by this method very desirable. As soon as a culture has been proved to be descended from an XX mother, that fact establishes the XX nature of each test culture descended from her, and accordingly each such set of test cultures furnishes about eight additional cultures in which all exceptions are known to be primary without the necessity of further tests. These test cultures, then, furnish highly valuable information on the frequency of primary non-disjunction. The other, and relatively minor consideration, was that these regular daughters were heterozygous for both eosin and miniature and their regular sons would furnish large amounts of data on the crossing over between these two loci. In order that there might be no confusion between the regular and the exceptional offspring in these tests, either in the female or in the male offspring, the tests were made by out-crossing to males carrying the dominant sex-linked mutation, bar. All of the exceptional daughters would then be wild type, the exceptional sons bar, the regular daughters bar (of the heterozygous type), and the regular sons would all be not-bar, but would be in four classes with respect to eosin and minature, as required by the known amount of crossing over between these loci.

Table 3 gives the results of the tests of several daughters from each of 23 of the cultures of table 1 that had given exceptions. While eight such cultures were started in each case, as expected, accidents or poor food or other unfavorable conditions cut down the final number in many cases.

In all, 162 daughters were tested which represented 23 original cultures of table 1. Of these sets of tests one only gave evidences of secondary non-disjunction, and this simply added a fourth proof of the XXY nature of the mother of culture 261. Of the twelve daughters of 261 seven gave secondary exceptions and five gave no exceptions. These five are accordingly included in the grand total of XX cultures of table 3. The remaining sets of cultures in each case proved that the mothers

TABLE 3

Offspring given by wild-type daughters from table 1 when out-crossed to bar males

	B	Regular sons				Exceptions	
		$w^e m$	+	w^e	m	+ ♀	*B* ♂
8	136	41	55	26	18	—	—
	86	40	27	15	14	—	—
	162	49	47	16	21	—	—
	60	21	15	12	9	—	—
	103	35	32	15	19	—	—
	125	39	24	21	21	—	—
Total	672	225	200	105	102	—	—
9	96	29	30	10	16	—	—
	86	32	29	17	15	—	—
	103	33	38	17	19	—	—
	129	40	35	16	17	—	—
	155	46	45	27	21	—	—
	168	49	50	22	29	—	1
Total	737	229	227	109	117	—	1
14	109	41	50	14	17	1	—
	109	32	27	8	11	—	—
	90	30	29	17	12	—	—
	144	50	43	23	24	—	—
	112	26	33	15	18	—	—
	72	22	16	11	10	—	—
Total	636	201	198	88	92	1	—
19	118	37	46	23	15	—	—
	124	37	44	15	11	—	—
	111	32	39	21	16	—	—
	86	25	38	10	10	—	—
	126	45	46	15	18	—	1
	35	14	14	3	5	—	—
	34	11	11	8	6	—	—
	100	26	28	16	19	—	—
Total	734	227	266	111	100	—	1
26	206	63	68	23	24	—	—
	154	44	45	26	20	—	—
	125	32	50	18	15	—	1
	103	38	47	18	19	—	—
Total	588	177	210	85	78	—	1
41	95	24	40	10	17	—	—
	151	47	41	21	21	—	—
	88	30	40	16	18	—	—
	119	42	34	19	27	—	—
	167	46	50	20	29	—	—
	79	27	22	9	11	—	—
41	155	58	50	19	21	—	—
	149	33	43	23	22	—	—
Total	1003	307	320	137	166	—	—
65	108	32	35	21	18	—	—
	103	38	28	15	16	—	—
	129	40	33	16	17	—	—
	106	44	38	23	19	—	—
	103	36	32	15	14	—	—
	75	32	24	8	14	—	—
Total	624	222	190	98	98	—	—
67	99	31	30	15	12	—	—
	70	31	33	12	19	—	—
	82	28	22	11	10	—	—
	101	33	39	16	18	—	—
	116	42	41	19	22	—	—
Total	468	165	165	73	81	—	—
81	217	67	77	31	32	—	—
	252	95	76	37	35	—	—
	221	81	92	33	36	—	—
	218	68	92	35	31	—	—
	166	50	56	26	25	—	—
	187	66	59	24	39	—	1
	194	70	62	29	36	—	—
	191	68	73	20	30	—	—
Total	1646	565	587	235	264	—	1
93	143	47	60	24	26	—	—
	98	50	34	18	16	—	—
	170	49	52	21	33	—	—
	223	81	72	27	36	—	—
	221	71	76	22	24	—	1
	190	70	67	26	29	—	—
	233	71	76	31	31	—	—
	205	74	68	20	27	—	—
Total	1483	513	505	189	222	—	1
106	203	59	84	37	33	—	—
	182	45	65	26	19	—	—
	239	70	74	25	25	—	—
	194	42	63	31	31	—	1
	158	52	61	24	28	—	1
	158	50	55	18	22	—	—
	171	40	49	30	26	—	—
	235	85	80	23	27	—	—
Total	1540	443	531	214	211	—	2

TABLE 3 (continued)

	B	Regular sons				Exceptions	
		w m	+	w^e	*m*	+ ♀	*B* ♂
109	293	106	109	54	49	—	—
	155	57	55	18	18	—	—
	216	77	82	32	35	—	—
	168	57	51	20	21	—	—
	128	43	46	17	22	—	—
	212	90	65	31	36	—	—
	156	70	55	16	29	—	—
Total	1328	500	463	188	210	—	—
117	192	63	59	19	34	—	—
	157	80	61	21	24	—	—
	227	85	100	35	36	—	—
	174	56	62	27	31	—	—
	252	96	89	37	37	—	—
	193	71	60	39	32	—	—
	129	47	40	19	23	—	—
	183	72	63	26	22	—	—
Total	1507	570	534	223	239	—	—
126	194	76	69	28	29	—	—
	159	56	65	20	32	—	—
	149	45	43	18	22	—	—
	65	24	17	11	7	—	—
	201	75	59	32	38	—	—
	186	60	45	18	19	—	—
	162	70	52	15	18	—	—
	252	92	87	41	41	—	—
Total	1368	498	437	183	206	—	—
137	206	64	54	37	24	—	—
	173	50	55	21	16	—	—
	177	54	78	24	27	—	—
	244	83	76	37	28	—	—
Total	800	251	263	119	95	—	—
144	219	66	59	17	27	—	—
	176	54	56	19	20	—	—
	196	61	61	31	34	—	—
	158	64	64	20	30	—	—
	150	50	53	21	18	—	—
	187	62	52	25	21	—	—
	181	81	68	25	24	—	1
Total	1267	438	413	158	174	—	1
155	227	97	73	47	40	—	—
	230	74	85	39	41	—	1
	226	82	71	38	44	—	—
	191	81	78	25	31	—	—
	69	31	26	10	12	—	—

	B	Regular sons				Exceptions	
		$w^e m$	+	w^e	*m*	+ ♀	*B* ♀
155	200	74	61	23	38	—	—
	219	65	75	31	35	1	—
	178	61	55	26	16	—	—
Total	1540	565	524	239	257	1	1
178	233	81	76	34	35	—	—
	132	37	41	19	24	—	—
	134	38	45	16	18	—	—
	165	51	54	23	22	—	—
	157	64	57	27	24	—	—
	112	43	46	22	19	—	1
	163	45	65	31	20	—	—
Total	1096	359	384	172	162	—	—
194	203	66	71	28	25	—	1
	156	44	45	25	20	—	—
	153	50	53	22	22	—	—
	163	55	56	23	27	—	—
	136	44	51	19	25	—	—
	114	43	38	16	18	—	—
	194	67	76	35	32	—	—
	189	58	68	23	25	—	—
Total	1308	427	458	191	194	—	1
228	203	68	84	26	31	—	1
	168	47	56	28	20	—	—
	239	83	88	29	42	—	—
	222	81	67	22	21	1	—
	176	73	58	13	24	—	—
	186	60	74	26	23	—	—
Total	194	412	427	144	161	1	1
245	135	57	46	23	21	—	—
	173	64	65	24	23	—	—
	155	55	49	23	24	—	1
	181	52	52	19	17	—	—
	121	40	38	26	21	—	—
	159	60	63	23	24	—	—
Total	924	328	313	138	130	—	1
246	197	55	71	26	28	—	—
	85	25	33	17	20	—	—
	226	73	70	36	33	—	—
	193	72	63	29	31	—	1
	197	80	66	27	27	—	—
	224	84	72	33	35	—	—
	196	77	56	24	29	1	—
	160	53	50	19	17	—	—
Total	1478	519	481	211	220	1	1

TABLE 3 (concluded)

	B	Regular sons				Exceptions			B	Regular sons				Exceptions	
		w^em	+	w^e	m	+♀	B♂			w^em	+	w^e	m	+♀	B♂
261	111	35	31	21	17	3	4	261	116	39	42	21	19	—	—
	86	26	28	15	13	1	3		91	29	28	16	13	—	—
(XXY)	73	30	26	14	16	2	1	(XX)	118	42	36	22	29	—	—
	112	37	40	18	22	8	6		83	26	34	13	17	—	—
	121	45	46	21	18	6	9		80	28	32	14	17	—	—
	104	33	40	18	19	3	3	Total	488	164	172	86	95	—	—
	132	46	51	22	23	5	6								
Total	739	252	262	129	128	28	32	Grand total*	24,429	8305	8268	3496	3674	4	14

* Exclusive of 261 (XXY).

of these cultures and of the parent culture (marked with a † in table 1) are all of the constitution XX and the exceptions are all primary.

With the completion of these tests we are in position to calculate the percentages of exceptions due to primary non-disjunction in table 1. There were 87 exceptions in a grand total of 47,545, or one in 546 flies; that is, 0.18 percent. Likewise the grand totals for the XX cultures of table 3 show that there were 14 patroclinous male exceptions and 4 exceptional daughters in a total of 48,190 flies or one in 2,677 flies; that is, 0.04 percent. The frequency of primary exceptions in these cultures was found to be about that in the experiments of BRIDGES and apparently lower than in my eosin miniature experiments, though whether the difference is large enough to be significant will be discussed in connection with further experiments on that point.

A very curious and significant relation came to light when the number of female and of male exceptions were compared. The number of such males ran far ahead of the corresponding females in both tables 1 and 3 and the total number of males, 84, was four times as great as the total number of females, 21. This feature will be discussed in a later section.

FREQUENCY OF PRIMARY EXCEPTIONS PRODUCED BY VERMILION FEMALES AND BY WHITE FEMALES

To determine whether the frequency of primary non-disjunction was different in different stocks, as might seem to be suggested by the difference between the results of BRIDGES's experiments (1 in about 2,000), the eosin miniature test (1 in about 550), and the test of regular daughters

(1 in about 2,600), a second and a third series of tests were carried out on the unrelated stocks of the sex-linked recessive mutants vermilion eye color and white eye color. Table 4 gives the results of the tests of 93 vermilion females from stock by out-crossing to wild males.

TABLE 4

Offspring given by vermilion females when out-crossed to wild males

Culture number	Regular offspring		Exceptions		Culture number	Regular offspring		Exceptions		Culture number	Regular offspring		Exceptions	
	+♀	*v*♂	*v*♀	+♂		+♀	*v*♂	*v*♀	+♂		+♀	*v*♂	*v*♀	+♂
1001	154	94	—	—	1039	146	135	—	—	1076	153	130	—	—
1002	110	106	—	—	1041	157	159	—	—	1077	80	77	—	—
1003	103	89	—	—	1042	167	131	—	—	*1078	143	157	—	1
1004	127	128	—	—	†*1043	116	121	—	2	1079	121	106	—	—
1006	159	174	—	—	†1044	101	61	1	—	1080	156	134	—	—
1007	114	98	—	—	1045	157	164	—	—	1081	132	126	—	—
†*1009	155	149	—	1	1047	115	114	—	—	1082	121	113	—	—
1010	159	138	—	—	1048	193	180	—	—	1084	145	82	—	—
†*1011	121	70	—	1	1049	174	115	—	—	1085	134	83	—	—
*1012	144	122	—	1	1050	199	160	—	—	*1086	119	115	—	1
1013	140	134	—	—	1051	39	24	—	—	1087	143	103	—	—
1014	123	110	—	—	1052	173	142	—	—	1088	106	91	—	—
1015	93	68	—	—	1053	157	139	—	—	1089	129	82	—	—
1016	157	135	—	—	1054	162	121	—	—	1090	103	108	—	—
†*1017	102	108	—	1	1055	157	144	—	—	1091	126	46	—	—
1018	180	113	—	—	*1056	144	156	—	1	†*1092	138	117	—	1
†*1019	136	104	—	1	1057	150	129	—	—	1093	82	58	—	—
1022	89	62	—	—	1058	195	162	—	1	*1094	141	130	—	1
1023	210	179	—	—	1059	152	181	—	—	1095	108	108	—	—
†1024	138	132	1	—	1060	210	157	—	—	1096	145	170	—	—
1025	110	86	—	—	1061	39	33	—	—	1097	106	101	—	—
1026	147	100	—	—	1062	145	153	—	—	1098	108	104	—	—
†*1027	79	54	—	1	*1064	117	92	—	1	1099	76	75	—	—
1028	164	103	—	—	1065	152	136	—	—	1100	138	130	—	—
*1029	144	160	4	6	1066	161	109	—	—	1101	141	101	—	—
1031	117	105	—	—	1067	171	154	—	1	1102	140	116	—	—
1032	200	177	—	—	1069	136	156	—	—	1103	126	104	—	—
*1034	92	93	—	1	1070	166	132	—	—	1104	163	102	—	—
1035	102	92	—	—	1071	138	125	—	—	1105	123	113	—	—
1036	135	115	—	—	1072	142	138	—	—					
1037	118	95	—	—	1074	120	111	—	—	Total‡	12,348	10,621	2	17
1038	108	108	—	—	1075	65	64	—	—					

‡ Exclusive of culture 1029.

The same sort of tests were carried out with these cultures as with those of table 1 in order to determine if any of the exceptions were due to secondary non-disjunction. It is immediately apparent that culture

1029 with ten exceptions (3.3 percent) is due to secondary non-disjunction. The sterility test was applied to the six exceptional males of culture 1029 and all proved fertile (table 5) as they should if of the supposed XY type.

TABLE 5

Offspring given by the six exceptional sons from culture 1029v when tested by wild females

No. 1029	+ ♀	+ ♂
1	122	119
2	97	100
3	134	129
4	108	99
5	152	142
6	88	92
Total....................	701	681

TABLE 6

The two kinds of results given by the regular daughters from culture 1029v when tested by bar males

No. 1029v	Regular daughters	Regular sons		Exceptions		Percent of exceptions
	Bar	*v* ♂	+ ♂	*v* ♀	*B* ♂	
1	110	48	56	2	3	2.3
2	136	71	63	5	4	3.2
3	78	39	36	3	2	3.1
4	150	73	80	6	3	2.9
5	102	59	57	3	5	3.5
6	131	63	78	4	7	3.9
Total........	707	353	370	23	24	3.1
7	108	65	48	—	—	—
9	129	63	76	—	—	—
10	79	51	43	—	—	—
11	127	68	72	—	—	—
12	58	27	29	—	—	—
Total........	501	274	268	—	—	—

The sterility test was applied to fifteen exceptional males from the other cultures of table 4, and in every case these males proved sterile (marked with an asterisk).

Tests of the constitution of the mothers of table 4 by means of tests for XXY daughters, were also carried out. In the case of culture 1029 six test cultures gave secondary exceptions (3.1 percent) while five gave no such exceptions (table 6).

Similar tests carried out with the regular daughters of nine other cultures of table 4 (marked with a †) gave in each case proof of the XX constitution of the mothers. In table 7 the sum of each set of such test cultures is given.

The vermilion XX females of table 4 yielded a total of 22,988 offspring of which 19 were exceptions, 17 males and 2 females. This represents one primary exception in 1209 offspring or 0.08 percent.

The tests of the daughters of culture 1029 (table 6) and of the other cultures (table 7) furnished 70 XX cultures with a total of 19,042 flies of which 12 were exceptions, 2 females and 10 males. This represents one primary exception in 1587 offspring or 0.06 percent.

TABLE 7

Summary of tests by out-crosses to bar males, of regular daughters from cultures of table 4 that gave exceptions

Test of culture	No. of ♀♀ tested	Regular daughters	Regular sons		Exceptions	
		B	+	*v*	*v*♀	*B*♂
1029	8	1148	529	536	—	2
1011	7	894	488	448	—	1
1017	8	1121	545	573	1	1
1019	8	1241	638	605	1	1
1024	6	817	404	379	—	1
1027	6	767	368	345	—	2
1043	7	1013	481	483	—	—
1044	8	1135	542	573	—	1
1092	7	986	461	467	—	1
Total........	65	9122	4456	4409	2	10

PERCENTAGE OF PRIMARY EXCEPTIONS GIVEN BY THE FEMALES OF THE WHITE STOCK

In a third experiment to test the percentage of primary non-disjunction, over a hundred white-eyed females were out-crossed to wild males (table 8).

The white-eyed females yielded 15 exceptions, 13 males and 2 females, in a total count of 21,773 offspring, which represents one exception in 1452 offspring or 0.07 percent.

The secondary matroclinous exceptions of cultures 261 and 1029 were out-crossed to wild males and gave the expected secondary exceptions in all cases (tables 9 and 10).

A summary and a comparison of the data from the various sources upon the frequency of the occurrence of primary exceptions is given in table 11.

TABLE 8

Offspring given by white females when out-crossed by wild males

No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions	
	+♀	*w*♂	*w*♀	+♂		+♀	*w*♂	*w*♀	+♂		+♀	*w*♂	*w*♀	+♂
2001	94	100	—	—	2038	107	109	—	—	2074	140	113	—	—
2002	80	94	—	—	2039	131	132	—	—	2075	128	104	—	—
2003	77	107	—	—	2040	90	94	—	1	2076	118	106	—	—
2004	105	105	—	—	2041	96	94	—	—	2077	117	103	—	—
2006	125	117	—	—	2042	130	130	—	1	2078	131	107	—	—
2007	111	103	—	—	2043	75	57	—	—	2079	116	117	—	—
2008	107	123	—	—	2044	72	73	—	—	2080	115	106	—	—
2009	106	101	—	—	2045	100	104	—	—	2081	109	104	—	—
2010	100	101	—	—	2046	130	87	—	—	2082	146	143	—	—
2011	79	81	—	—	2047	101	89	—	—	2083	116	142	—	—
*2012	90	86	—	1	2048	156	130	—	—	2084	143	94	—	—
2013	76	63	—	—	2049	92	91	—	—	2085	132	161	—	1
2014	96	101	—	—	2051	115	106	—	—	2086	149	127	—	—
2015	89	72	—	—	2052	120	110	—	—	2087	111	93	—	—
2016	84	61	—	—	*2053	131	120	1	1	2088	115	121	—	—
2018	114	98	—	—	2054	73	87	—	—	2089	107	114	—	—
2019	95	90	—	—	2055	141	06	—	1	2090	100	103	—	—
2020	145	144	—	—	2056	130	127	—	—	2091	156	156	—	—
2021	108	52	—	—	2057	165	148	—	—	2092	151	112	—	1
2022	111	55	—	—	2058	124	122	—	—	2093	111	111	—	—
2024	109	99	—	—	2059	123	127	—	—	2094	149	128	—	—
2025	106	102	—	—	2060	130	112	—	—	2095	168	149	—	—
2026	89	88	—	—	*2061	155	125	1	1	2096	140	122	—	—
2027	57	55	—	—	2062	117	92	—	—	*2097	107	115	—	2
*2028	142	119	—	1	2063	152	139	—	—	2098	129	126	—	—
2029	104	87	—	—	2064	112	93	—	—	2099	155	128	—	—
2030	125	112	—	—	2065	115	96	—	—	2100	134	121	—	1
2031	93	95	—	—	2066	111	108	—	—	2101	102	110	—	—
2032	74	111	—	—	2067	103	81	—	—	2102	122	118	—	—
2033	97	89	—	—	2068	123	113	—	—	2103	103	104	—	—
2034	88	79	—	—	2069	126	147	—	—	2104	119	91	—	—
2035	125	100	—	1	2070	80	66	—	—	2105	141	96	—	—
2036	91	77	—	—	2071	70	82	—	—					
2037	69	76	—	—	2073	71	75	—	—	Total	11,308	10,450	2	13

RELATIVE NUMBER OF FEMALE AND MALE EXCEPTIONS

One of the most puzzling facts disclosed by the foregoing experiments is the uniformly lower frequency of the female exceptions. Out of a

TABLE 9

Offspring given by the three exceptional daughters from culture 261 when tested out by wild-type males

No.	Regular offspring		Exceptions		Percent of exceptions
	+♀	w^em♂	w^em♀	+♂	
261a	100	94	2	3	2.6
261b	125	98	1	1	0.9
261c	120	85	3	3	2.9
Total.........	345	277	6	7	2.09

TABLE 10

The offspring given by four vermilion exceptional females when out-crossed by wild males

No.	Regular offspring		Exceptions		Percent of exceptions
	+♀	*v*♂	*v*♀	+♂	
	123	140	6	9	5.4
	94	101	5	4	4.4
	139	143	6	8	4.7
	108	118	6	5	4.6
Total.........	464	502	23	26	4.8

TABLE 11

Summary of data upon the frequency of primary non-disjunction

Series	Total offspring	Total females	Total males	Total exceptions	Total ♀ exceptions	Total ♂ exceptions	Ratio of total exceptions	Ratio of ♀ exceptions	Ratio of ♂ exceptions	Percent of exceptions	Percent of ♀ exceptions	Percent of ♂ exceptions
BRIDGES....	20,484	10,635	9,849	12	5	7	1:1707	1:4097	1:2926	0.06	0.02	0.03
w^em.......	47,545	25,021	22,524	87	17	70	1:546	1:2800	1:679	0.18	0.04	0.15
v..........	22,988	12,350	10,638	19	2	17	1:1209	1:11494	1:1352	0.08	0.009	0.07
w..........	21,773	11,310	10,463	15	2	13	1:1452	1:10886	1:1675	0.07	0.009	0.06
w^em test cases.....	48,190	24,433	23,757	18	4	14	1:2677	1:12047	1:3442	0.04	0.008	0.03
v test cases..	19,042	9,625	9,417	12	2	10	1:1587	1:9521	1:1904	0.06	0.01	0.05

total of 151 exceptions yielded by the eosin miniature, vermilion, and white, females, and the test cases, only 27 were females whereas 124 were males (table 11).

This difference in the numbers of male and female exceptions cannot be explained by simple primary non-disjunction which results from a delayed reduction for the X chromosomes; for when the polar body is formed the entangled X's pass together to one or to the other pole giving two classes of eggs, XX and O, in equal numbers. These eggs when fertilized by the X and Y sperm, present in equal numbers in a normal male, would result in equal numbers of XO male and XXY female exceptions.

Neither will secondary non-disjunction help out, for BRIDGES found that the percentage of secondary exceptions is the same in the females as in the males, which is in sharp contrast to the condition here found.

This excess of males might be accounted for by assuming that the two X's are extruded into the polar body more frequently than retained in the egg nucleus. There seems no *a priori* reason why this should be so, and DONCASTER (1914) reports that his cytological investigations of a somewhat similar sex disturbance in Abraxas show apparently that the direction taken by the sex chromosomes on the polar spindle is at random.

Another explanation that appears to have more adequate support, is that the greater frequency of O eggs is due to some type of "elimination" of the X chromosome that would normally remain in the egg at the maturation division. MORGAN and BRIDGES (1919) report the occurrence of numerous gynandromorphs in Drosophila, the explanation of which they have proved to be an "elimination" of an X chromosome, occurring at an early cleavage division when two X's divide. They show that in this way an XX zygote may give rise to a nucleus with only one X, at the first, second, or some later cleavage. The parts supplied by the descendants from this one-X nucleus are accordingly male, and may constitute a half, a quarter, or some othe frraction of the individual, depending upon the cleavage division at which the X is eliminated. The parts descended from the original XX type of nuclei are female. If lagging were to occur at the reduction division causing the "elimination" of a whole X chromosome, no-X or zero eggs would be produced whenever the mate of the lost X went out into the polar body. The fertilization of such no-X or zero eggs would produce XO zygotes which would account for the excess of exceptional males.

The formation of O eggs by such a loss of the X chromosome that would normally remain in the eggs gives the same kind of end result as does primary non-disjunction. We may suppose that the initial step in this process is some difficulty in the separation of the synapsed X chromosomes. Either they had become mechanically intertwined to such a degree or in such a fashion that disjunction is more than ordinarily difficult; or the chemical or other forces that had held them in junction were slow to relax in these particular cases. Besides local environmental conditions that would favor such a delay, it seems probable that in the case of the eosin miniature stock there is present a specific chromosome alteration that makes such a condition more frequent.

There is still a third way of explaining the formation of more O than XX eggs. Extreme lagging of the X chromosomes on the middle of the polar spindle, from whatever cause, would mean that the daughter nuclei would be formed without the inclusion of an X in either. Both X's would be eliminated. All such eggs would be of the O type, and when fertilized would lead only to patroclinous, XO exceptional sons. These primary male exceptions would constitute an excess in proportion to the frequency of the occurrence of this kind of elimination.

There is supporting evidence for the occurrence of "elimination" in forms other than Drosophila. Thus in bees the occurrence of gynandromorphs in which the male parts are sometimes maternal and sometimes paternal are fully explained by elimination, but are not both explainable on any of the former theories of gynandromorphism. Some of the gynandromorphs in some of the moths and butterflies can be explained as due to elimination. In certain nematodes (e.g., *Rhabditis nigrovenosum*) the production of the sexual males depends upon the elimination of an X from cells which were of the hermaphrodite or female type. These considerations made it probable that the excess of primary male exceptions is due to the elimination of both X's from the egg. The cases of female exceptions and a corresponding number of male exceptions are due to typical primary non-disjunction followed by the inclusion of both X's in the egg or in the polar nucleus.

This conception of the case brings into line the surprising fewness of females of the XXY type among females picked at random from culture bottles. Only two cultures among the thirty-two tested gave secondary exceptions, which is in conformity with the very rare formation of XX eggs by primary non-disjunctions.

STERILITY OF THE XO MALE PRODUCED BY PRIMARY NON-DISJUNCTION

As already stated, BRIDGES had tested fifteen primary exceptions and had found them all to be sterile. In the tests of the nature of the exceptions in the cultures of tables 1, 4, etc., I tested a total of fifty-four other such males. In many of these cases there was other and independent evidence of the primary nature of these exceptions. It was hoped that offspring might be obtained from these XO males in order that a race lacking the Y chromosome might be secured and made the basis of further investigations. Accordingly, exceptional efforts were made to favor fertilization. The XO males were entirely normal in appearance, and, as was carefully tested, were normal in their sexual behavior. Courtship and copulation were normal in method and in the time relations (described by STURTEVANT 1915). Old XO males copulated more readily, and copulation was hastened by the odor from previous copulations, as is the normal condition. The females that had been copulated with by XO males laid eggs, which however in no case developed, despite unusual care. Food conditions were kept favorable by the introduction of larvae which prevented the undue growth of bacteria, yeast, and moulds. Larvae from white-eyed flies, or else, fertilized white females from stock were used so that any true offspring of the XO males could be detected by the different eye color. All these efforts failed, and it became apparent that the sterility of the XO males was established.

CAUSE OF STERILITY OF XO MALES

The first problem attacked in an effort to find the cause of such sterility was whether or not the XO male injects sperm during copulation. An examination of the spermathecae dissected from females after such copulation showed that no sperm was present, while copulations by normal XY males introduced an abundance of motile sperm.

Preparations of the genitalia of XO males were made after treatment with KOH which removed the non-chitinous materials. No difference could be detected between these preparations and others of normal fertile males.

Upon dissection of XO males testes were found to be present and to be of normal shape and color, though probably of smaller size. When these testes were teased open, bundles of sperm appeared as in the normal testes; but these bundles remained compact instead of separating out as do normal bundles, and when the bundles were artificially separated it was found that the sperm of the XO male are non-motile. It was found also that considerably less sperm was produced by the XO male than normal. Many such dissections and comparisons were carried out, and it became apparent that the immediate cause of the sterility was the non-motility of the relatively scanty sperm.

CYTOLOGICAL EVIDENCE OF THE OCCURRENCE OF XO MALES

The genetic evidence of BRIDGES and myself leaves no escape from the conclusion that the patroclinous exceptional sons resulting from primary non-disjunction were of the constitution XO instead of XY. It was anticipated that a cytological demonstration of that point would be my most difficult task.

The cytology of Drosophila males has always presented many serious difficulties; Miss STEVENS, for example, made preparations of some 2,000 males with only partial success in the description of the group. Several other workers have made preparations. The best thus far secured are those of BRIDGES, by which he was able to demonstrate the J shape and the larger size of the Y chromosome. In the case of the XO males I was under the further serious handicap of strictly limited material. To secure one male required the examination of 1,000 flies on the average.

BRIDGES had made preparations of five of his XO males, but these showed the same condition as my early preparations, namely, a few bundles of sperm and very little spermatogonial or spermatocyte tissue. These had been made from males that had been tested for their sterility to be sure of their XO character. But males so old as this have nearly finished the spermatogonial and spermatocyte divisions, and are unsuitable, as we found, for cytological examination.

The next step was to omit sterility tests and to make preparations of the freshly hatched exceptional males, relying upon the tests of sisters to prove their XO nature. This procedure yielded little better results than before; for although the fixation (strong Flemming) and the staining (iron haematoxylin) appeared satisfactory, divisions were not found. These preparations showed slightly greater amounts of spermatogonial tissue than the old males had, but from the fact that the most successful preparations of normal males made by BRIDGES were of larvae, in which adult sperm were already present, it was apparent that the earliest stage at which I could distinguish exceptions must be used. There was unfortunately no sex-linked larval character on hand that could be used, so that characters which showed in the pupa were chosen. Matings were made between eosin miniature bar females (eosin miniature females had given the highest percentage or primary exceptions) and wild males. The character bar eyes was used because it was the one that could be earliest and most surely detected in the pupa. Any male pupa with a full round not-bar eye would be the desired exception. Five such males were ultimately secured. In fixing them an important change in technique was used, namely, to dissect out the testes free from all fat-bodies which interfere with the action of osmic fixation.

In four out of the five, pupal figures were found that were analysable. In two or three there were a number of mitotic figures that were good and there were a few that can be regarded as demonstrative.

The point about which there is no question is that in none of the figures of these pupae is there a Y chromosome visible. BRIDGES had found that the Y chromosome, more than any other, tended to stand out clearly. It was usually well isolated and remained condensed so that it stained deeply and sharply. The difference in length of the limbs was clearly defined and enabled him to distinguish the J-shaped Y from the equal-armed second and third chromosome V's.

The spermatogonial groups are of greatest value but are very rare. Probably a half-dozen were examined of which three or four were fairly clear. Two (3 and 4) in one pupa were demonstrative. In the spermatogonial groups there was an unpaired rod,—the X,—two pairs of V's, and a pair of spherical chromosomes. These two XO figures should be compared with XY figures from normal males, of which an example is given in figure 2 (a previously unpublished figure furnished by BRIDGES).

There were no satisfactory first-spermatocyte figures, but there were many good "interkineses" (plate 1, figures 5–14) and many "second" divisions (plate 1, figures 15–19) and some spermatid cells (figures 20–21). Interkinesis groups were expected to be of two types equally numerous: one type possessing an X (figures 5–14) was frequent and clear; the other possessing no X (figures 18, 19) was infrequent and uncertain.

The second-spermatocyte divisions were likewise expected to be of two types. In one type (plate 1, figures 15, 16, 17) there is the normal 4-chromosome group, including an X, approaching each pole. This type was clear and unmistakable. In the other type there are only three chromosomes,—two V's and the spherule,—with no X chromosome, but in none of the few cases observed is it certain that an X has not been lost in sectioning.

The spermatids were likewise expected to be of two types corresponding to the products of the second divisions. The X type was clear, but again the type in which two V's and a spherule are present without a sex-chromosome could not be clearly demonstrated.

To sum up, the no-X type of interkinesis group, the no-X type of second-division and the no-X type of spermatids that would be expected to result from reduction divisions in an XO male seemed not to be as numerous as the corresponding X type. Indeed, there is some question as to whether any were really present. Furthermore the number of nuclei in the "interkinesis" stage was exceptionally large; and no certain first-maturation divisions were found. Also it was observed that in the "interkinesis" often the small chromosomes were not in contact with each other as they should be if they were sister chromosomes about to undergo an equational separation. These findings may mean that one division is suppressed and that in the stage labeled "interkinesis" the V's attached by their apices represent unreduced autosome pairs while the double-length rod is the X with an equational split. The single maturation division following this stage would give each of the two resulting spermatids the full haploid number of chromosomes.

There are two general points in connection with the figures that should be mentioned. BRIDGES (1916) had observed that in the gonial figures of males and females there was a striking demonstration that the forces which caused the splitting of a chromosome into two daughter chromosomes and that responsible for the drawing of those daughters to the poles were independent. The metaphase chromosomes begin to split and separate at the free outer ends and the separation travels progressively inward until the chromosomes are in contact at the apex only of the V's and at the inner end only of the X. These daughter parts seem to repel each other and to stand out so that an autosome presents two V's with their apices joined and the free limbs directed poleward. Afterwards the union between the apices is dissolved and the apices travel toward the poles so that the relative positions of the daughter V's are reversed. This repulsion is seen in the clearest fashion in the "interkinesis" figures where the X chromosome opens out at the ends to form a V and the large autosomes become double V's with their free arms directed to the four angles of a tetrahedron, as is clearly shown in plate 1, figure 11 and others.

GYNANDROMORPH "51"

A gynandromorph appeared (July 18, 1915) in culture 51, which was a cross between an eosin miniature female and a wild male. The abdomen was female in size, shape and coloration, and appeared to contain a pair of ovaries. The genitalia were likewise female. The right wing was wild-type (female) and the right half of the thorax was large (female). The left wing was minatµre and the left side of the thorax was smaller (male) in consequence of which the abdomen was twisted to the left. The bristles also were smaller on the left side of the thorax. The division of the thorax into male (left) and female (right) sides did not extend

LEGEND FOR PLATE 1

The figures in this plate were drawn at table level; tube length 160 mm; Zeiss compensating ocular 12X; and Zeiss apochromatic 1.5 mm oil immersion objective, N. A. 1.30. The figures were then enlarged 2½ diameters, and in reproduction were reduced in the ratio 3:2. The resulting magnification is 5,115 diameters.

Figure 1 (furnished by BRIDGES) is an oögonial plate of a wild female. Two X chromosomes are present as rods. This figure is from a freshly hatched mature fly; the rest of the figures are from pupae.

Figure 2 (previously unpublished figure furnished by BRIDGES) is a spermatogonial plate of a wild male. The Y chromosome has its characteristic unequal-armed J shape. This figure shows a late metaphase group in which the chromosomes are already split; the same split condition is seen in 3 and 4.

Figures 3 and 4 show late-metaphase spermatogonial groups from an exceptional (XO) male. Both are from one individual. In these groups there is an unpaired rod, the X, two pairs of autosomal V's already split, and a pair of spherules, the "fourth" chromosomes.

Figures 5–14 from XO males show a few of many good "interkinesis" groups. In "interkinesis" the X chromosome, ordinarily rod-like, splits into a V which opens out into a double-length rod. Likewise the autosomal V's resolve into two V's which remain attached at their apices with the free limbs directed toward the corners of a tetrahedron.

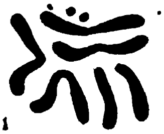
Figures 15, 16, 17, 18, 19 show "second-spermatocyte" division groups. Figures 15, 16 and 17 contain an X, while 18 and 19, appear to be of the other expected type in which there is no X. The nuclei of 18 and 19, may be cut and incomplete, so that no great weight is to be attached to them. There were many "interkinesis" groups, "second-spermatocyte" divisions, and some spermatid groups showing an X present. None showed a Y. Several might well be without an X but none of these latter were conclusive.

Figures 20 and 21 are polar views of two spermatid groups that also appear to be of the no-X type. Both these divisions were cut through the mid-region between the groups, and while no X was detected it can not be certain that no X was present.

Figures 22–24 show giant multinucleated cells. These represent final spermatogonial cells and spermatocytes that failed to form separate cell boundaries; or more probably they have resulted from the coalescence of separate cells in the same stage of development.

In general, the most important point about the figures examined is that in none of them is there a Y chromosome visible. BRIDGES had found that the Y chromosome, more than any other, tended to stand out clearly, was usually well isolated, and remained condensed so that it stained deeply and sharply; this is seen especially well in figure 2. There can be no mistake about the absence of such a Y chromosome from the groups of these slides.

SAFIR, SHELLEY R.. NON-DISJUNCTION IN *Drosophila melanogaster* Plate 1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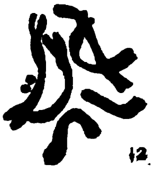
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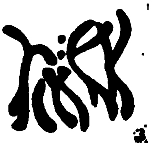
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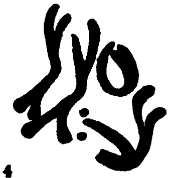
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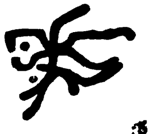
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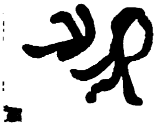
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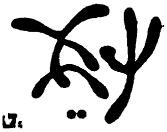
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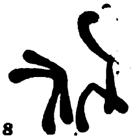
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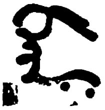
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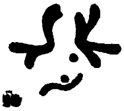
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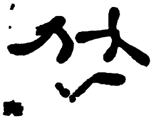
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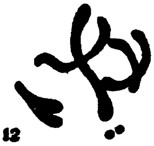
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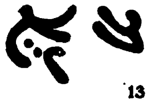
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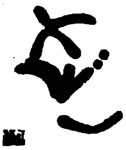
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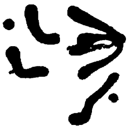
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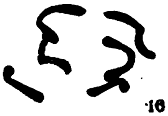
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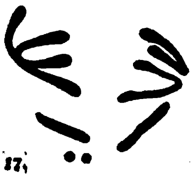
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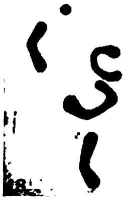
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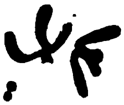
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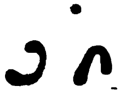
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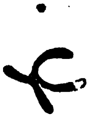
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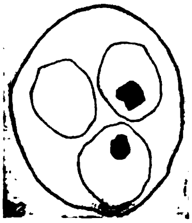
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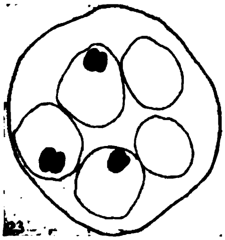
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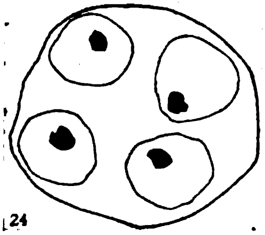
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through to the ventral side for both fore-legs bore sex combs and were therefore purely male. The head was small and both eyes were eosin of the male color. The head was therefore entirely male.

MORGAN and BRIDGES (1919) have shown that the usual cause of gynandromorphism in Drosophila is the "elimination" (at an early cleavage division) of one of the two X chromosomes of a female. The parts that descend from the cell that receives only one X become the male parts of the gynandromorph, and the other cells that retain the original two chromosomes give rise to the female parts. An egg bearing an eosin miniature X was fertilized by a sperm bearing a wild-type X. The zygote was of the same type as the regular daughters expected from the cross. But at an early cleavage division (probably the second in this case) one of the two X's that come from the division of the paternal wild-type X, failed to be included in the daughter nucleus, probably being entangled at the mid-plate of the division. This cell received a full complement of autosomes but only one X chromosome—the maternal eosin miniature X. All the parts that developed from this cell (the head, the forelegs, and half the thorax with its wing) were male and were at the same time eosin and miniature. The remaining parts were female (XX) and showed only wild-type characters, since the eosin and miniature carried by the maternal X are both recessive, if both gonads arise from a single isolated cell they should always be either male or female, in this case female.

SOMATIC MUTATION FROM EOSIN TO WILD-TYPE

In a pure stock culture of eosin miniature there appeared (August, 1915) a single male whose left eye was completely red and whose right eye was eosin with a vertical bar of red across the middle. This appearance of red in a pure stock of eosin means a reverse mutation from eosin to the wild type or to an allelomorph not distinguished from the wild type. The peculiar distribution of the red indicates that the mutation occurred in the early embryonic stage in the somatic tissue. This fly was tested by breeding and behaved as does a pure eosin miniature.

BUFF, A SIXTH MUTANT ALLELOMORPH OF WHITE EYE COLOR

In cultures No. 55 and 188 of the eosin miniature series of tests there appeared, (July 28, 1915), besides the expected classes, two and eight females which had long wings like their wild-type sisters but whose eye color, instead of being red, was lighter than that of standard eosin females and only a little darker than that of eosin males. These light-eyed females were put into a culture bottle where they produced offspring, having evidently been fertilized by their eosin miniature brothers. Unfortunately little attention was paid to the offspring of these females. Apparently half of the males were eosin and half lighter than eosin having a pale cream or "buff" eye color. Most of the buffs were not miniature but a buff miniature was selected for breeding in order to take advantage of the miniature in placing the new mutant.

The buff miniature male was out-crossed to a wild female and gave only red daughters (195) and sons (187) which shows buff to be recessive. About half the F_2 males, but none of the F_2 females, showed the buff color which is thus shown to be due to a sex-linked gene. There was 35.2 percent of crossing over between buff and miniature which suggested that the locus of buff was at least very close to that of eosin. Not one of the sons was eosin in color, which showed that buff was probably not eosin plus a diluter or specific modifier. In subsequent experiments (SAFIR 1916) a total of 14,811 flies involving the linkage of buff and miniature was raised. The crossover value was 35.5 and no eosin appeared. In back-cross experiments involving yellow, buff, and miniature, 7,537 flies were raised which gave a position for buff 1.9 units to the right of yellow. The early yellow white data gave 1.1 as the distance of white or eosin to the right of yellow, but later and better data show that the distance is probably nearer 1.6 which agrees well with the 1.9 here obtained for yellow buff.

Buff males were out-crossed to white, eosin, and cherry females and in each case the F_1 females were not red but were intermediate in color between the parental colors. The formation of compounds together with the practical identity of linkage relations between buff and white shows that buff is an allelomorph of white.

The buff female is of the same intensity of color as the male, as is the case of all the white allelomorphs except eosin.

The white allelomorph series is wild-type, white, eosin, cherry, blood tinged, and buff.

It is apparent that the original light-colored females were buff-eosin compounds and that the buff mutation had occurred in the wild-type father.

GIANT OR MULTINUCLEATED CELLS IN THE TESTES OF THE XO MALES

It had been found that the testes of XO males contained relatively few packs of sperm as compared with a normal male. The reason for this became apparent when the sections of the testes were examined. The spermatogonial cells were approximately normal in numbers but the final spermatogonial cells and spermatocytes quite frequently failed to form separate cell boundaries.

A rounded syncytium was thus formed that contained two, four, or eight nuclei (plate 1, figures 22, 23, 24). It seems probable that in some cases the entire contents of the small cysts were included within a common cytoplasmic body. It was observed that the nuclei of many of these giant cells seemed abnormal or degenerating. It is believed that these giant cells often die and disintegrate without the formation of spermatozoa. In other cases it seems probable that these giant cells have formed the bundles of non-motile non-separable spermatozoa to be found in the lumen of the testis.

According to the account of WODSEDALEK (1916) such giant spermatogonia and spermatocytes exist in the mule, and their degeneration precludes the formation of spermatozoa and leads to the sterility of the mule.

SUMMARY

In *Drosophila melanogaster*, as in other animals, in normal reduction divisions of XX females the two X chromosomes disjoin from each other and pass to egg and to polar body, respectively, so that each mature egg has one X chromosome.

In his work on non-disjunction BRIDGES found that:

(1) Occasionally the two X chromosomes do not disjoin (primary non-disjunction) but are both retained in the egg (XX egg) or are both extruded to the polar body (zero or no-X egg).

(2) The fertilization of the XX egg by an X sperm gives a non-viable XXX individual. An XX egg fertilizer by a Y sperm gives an XXY matroclinous "exceptional" daughter, which in turn gives "exceptions" due to the action of the supernumerary Y (secondary non-disjunction).

(3) The fertilization of a no-X egg by a Y sperm gives a non-viable zygote (YO). A no-X or zero egg fertilized by an X sperm gives an XO individual which is a patroclinous "exceptional" son.

My further work on primary non-disjunction has led to the following results:

1. The constitution of primary exceptional sons has been proved to be XO by direct cytological examination. Their spermatogonia were found to contain an unpaired X chromosome. No Y chromosome was present. The products of the first, or reductional, divisions were expected to be

nuclei containing an X and nuclei free from an X. The latter type was certainly not present. Other aberrations also were present that suggest that the one maturation division was wholly or frequently suppressed.

2. XO males are indistinguishable in sex-linked characters, in somatic characters, in genitalia, and in mating behavior from XY males, but are absolutely sterile (about 69 tested, including about 15 tested by BRIDGES).

3. The spermathecae and oviducts of females contained no sperm after copulation with XO males.

4. In the testes of XO males were found only a few bundles of sperm and these bundles did not readily break up into individual spermatozoa.

5. The spermatozoa were non-motile.

6. A cytological examination of the testes showed that there were present multinucleated giant cells; the cytoplasm of many cysts consisted of a common rounded mass with no cell division corresponding to the nuclear divisions.

7. The non-motility of the sperm and the compactness of the bundles seem to be a result of the syncytial nature of the cysts.

8. The percentage of primary exceptions observed by BRIDGES was 0.06 (12 in a total of 20,484). The percentage of primary exceptions produced by eosin minature females was 0.18 (87 in a total of 47,545); by vermilion females the percentage was 0.08 (19 in a total of 22,988); by white females, 0.07 (15 in a total of 21,773).

9. The above exceptions were proved to be primary by three tests: (a) The number per culture was too low to be due to secondary non-disjunction; (b) exceptional males were usually tested and all tested were found to be sterile and therefore XO in constitution; (c) sets of eight regular females were tested from each culture giving exceptions and these were proved to be free from Y chromosomes.

10. The sets of tests of daughters gave in the case of the eosin miniature line 48,190 additional offspring of which 18, or 0.04 percent, were primary exceptions. The vermilion-series daughter tests furnished 19,042 offspring of which 12, or 0.06 percent, were primary exceptions.

11. The percentage of exceptions produced by the eosin miniature strain (0.18 on the basis of 47,545 flies) was approximately 3 times as high as in other experiments which all gave nearly the same percentages and totaled 64 in 111,993 flies, or 0.06 percent. It is probable that the eosin miniature strain contained a mutant gene that favors the production of primary exceptions. This gene would be a recessive since the normal percentage of primary exceptions was given by the tested daughters, which were the F_1 from an out-cross of that line.

12. The primary exceptions reported by BRIDGES were 5 females and 7 males. In my tests (and in other cases by BRIDGES) there is a marked excess of males, viz., in the eosin miniature direct tests 17 females and 70 males, in the vermilion tests 2 females and 17 males, in the white tests 2 females and 13 males, in the daughter tests respectively 4 females to 14 males, and 2 females to 10 males. In all (including BRIDGES's) there have been 32 females to 131 males, or a ratio of 1:4.

13. The probable explanation of this excess of male primary exceptions is that they have resulted from a type of "elimination" at the reduction division. The two X chromosomes failed to disjoin from each other, and lagging upon the center of the spindle were caught by the formation of the mid-body, and were prevented from becoming part of either nucleus. All eggs preceded by such non-disjunctional elimination would be no-X and would produce only XO males.

In conclusion, I wish to acknowledge my indebtedness to Prof. T. H. MORGAN for his interest in the progress of my work and for his many valuable suggestions, and especially to Dr. C. B. BRIDGES for his interest in supervising the experiments and in the writing of this paper.

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GENETIC STUDIES ON *DROSOPHILA SIMULANS*. I. INTRODUCTION. HYBRIDS WITH *DROSOPHILA MELANOGASTER*[1]

A. H. STURTEVANT
Columbia University, New York City

[Received July 6, 1920]

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INTRODUCTION

Many attempts have been made to hybridize different species of Drosophila, but hitherto all but the combination to be described here have been unsuccessful. The present case is itself only to be considered a partial success, since the hybrids have so far all been completely sterile. Furthermore, since the two parent species are extremely similar and probably have identical chromosome groups, the data that are to be obtained from the study do not throw as much light as might be wished on many of the problems concerning the nature of the specific differences found in the genus Drosophila. Nevertheless, the investigation has led to interesting results bearing on such subjects as interspecific sterility, parallel mutations, chromosome maps, and sex determination.

DESCRIPTION OF *Drosophila simulans*

Drosophila simulans STURTEVANT (1919) is very similar to the well-known *D. melanogaster* Meigen (*ampelophila* Loew), and has been confused with it until recently. There is only one satisfactory character by which

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[1] Contribution from the CARNEGIE INSTITUTION OF WASHINGTON.

the two species may always be separated; viz., the external male genitalia (figures 1 and 2). In living or relaxed pinned material the posterior process (P) of the genital tergite appears like a clam-shell in *D. simulans*, and like a small hook in *D. melanogaster*. The eggs (figures 3 and 4) are also characteristically different, but are more variable than the male genitalia, besides being less convenient to obtain. In addition to these characters, the cheeks are a little narrower and the eyes a little larger in *simulans*, and in general the newly hatched specimens have a more definite dark streak down the dorsal surface of the thorax (mesonotum) than has *melanogaster*. With a little experience one can usually separate most of the individuals in a mixed stock by the use of these characters. In addition there are other still more indefinite differences, that are observable when large series are compared. *Drosophila simulans* averages somewhat stouter in abdominal shape and darker in color than *melano-*

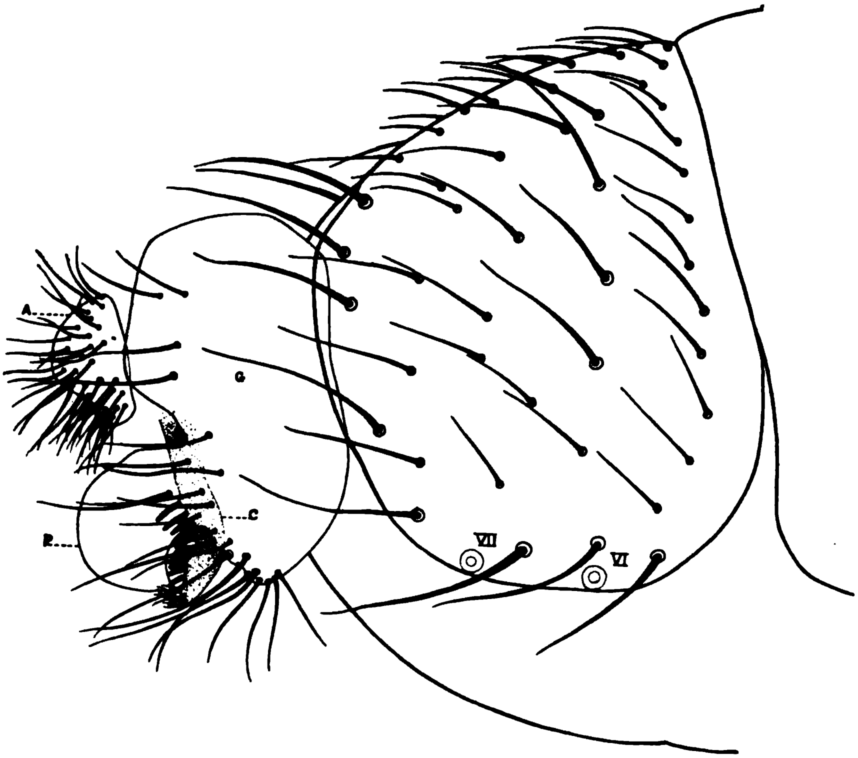

FIGURE 1.—External male genitalia of *Drosophila simulans*. From a specimen cleared and mounted in balsam. A, anal plate; C, clasper; G, first genital tergite; P, posterior process of genital tergite; VI, VII, abdominal spiracles on fifth abdominal tergite.

gaster. In mixed laboratory cultures *simulans* is apt to be smaller, though in pure cultures or in wild material there is no appreciable size difference.

Though experience will give one the impression that the two forms are distinct and should be classed as "good species," they are so close that a technical description of *D. melanogaster* or a drawing of the style familiar to geneticists would pass equally well for *D. simulans*. So far as I have been able to discover, the mating and breeding habits and the length of the life-cycle are identical. Dr. C. W. METZ reports, after a preliminary study, that the chromosome groups are probably also identi-

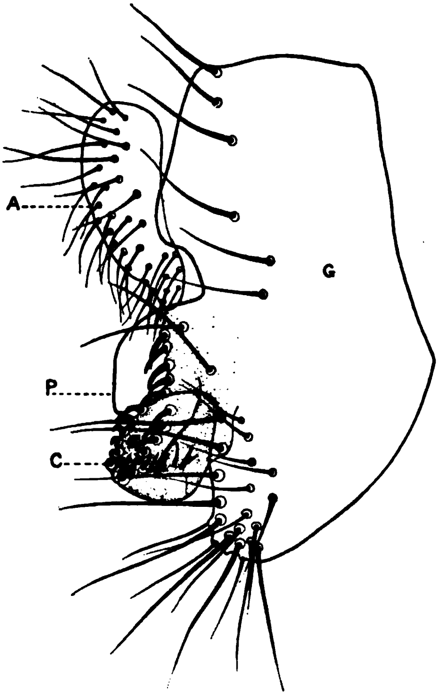

FIGURE 2.—External male genitalia of *Drosophila melanogaster*. Abbreviations as in Figure 1.

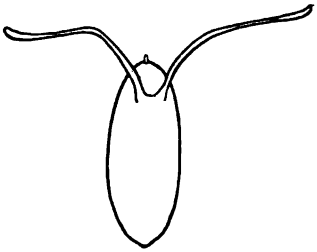

FIGURE 3.—Egg of *Drosophila simulans*.

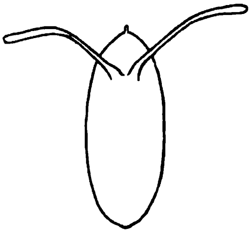

FIGURE 4.—Egg of *Drosophila melanogaster*.

cal. I am unable to distinguish the larvae, the pupae, or the female genitalia. The structure of the male genitalia and the sterility of the hybrids remain as the only valid grounds for making a specific distinction; but I think there can be no question that these grounds are sufficient.

DISTRIBUTION OF *Drosophila simulans*

I described *D. simulans* from stock collected at Lakeland, Florida. I have also seen specimens from New Hampshire, New York, Minnesota, Virginia, Georgia, Alabama, Costa Rica, Panama, and the Brazilian states São Paulo and Matto Grosso. Rather extensive collections from the Pacific coast and from the West Indies have so far not been found to contain any *simulans*, and it has not appeared in the Old World material that I have seen. *Drosophila melanogaster*, on the other hand, is evidently a cosmopolitan species. I have seen undoubted specimens, determined by examination of male genitalia, from American localities scattered from Nova Scotia to Oregon, California, Florida, Porto Rico, Costa Rica, Chile, and Brazil; and from Holland, Spain, New Zealand, Australia, and Hawaii. It is also recorded from Africa, India, and elsewhere.

Evidence has been presented by JOHNSON (1913) that indicates that *D. melanogaster* was introduced into this country from the tropics, and became common soon after 1870. The arguments advanced by JOHNSON are equally applicable to *simulans*, so far as they indicate the rarity or absence of the species before 1870, since the two forms have practically identical habits, and are so similar in appearance that even when JOHNSON wrote they had never been distinguished. The period at which *simulans* was introduced remains doubtful. It seems certain that QUACKENBUSH (1910) had specimens of it, derived from a stock collected at Woods Hole, Massachusetts, in 1908, and this is the earliest record known to me. The earliest specimen that I have actually seen was collected in Florida in 1912. It is quite probable, however, that an examination of large series of earlier material would show the species to have been established much earlier. The present distribution, outlined above, indicates that the species must have come from South or Central America.

DISCOVERY OF *Drosophila simulans*

At present *Drosophila simulans* and *D. melanogaster* are about equally common around New York City, in Florida, in Alabama, and in southwestern Brazil. Since the two are distinguishable only with some practice, and since a fresh wild stock usually contains both, it seems very surprising that *simulans* was not accidentally discovered long ago in the course of genetic experiments with *melanogaster*. It must have been in the laboratory many times, but seems to have led to unexpected results only two or three times. The curious "unisexual broods" recorded by QUACKENBUSH (1910) were surely hybrid broods. Professor E. A. ANDREWS reported in correspondence some years ago that he had had difficulty in crossing a mutant race of *melanogaster* to a wild race collected at Baltimore, Md., and it now seems probable that the wild race was *simulans*. Finally, Mr. A. M. BROWN, in the COLUMBIA laboratory, obtained anomalous results with a wild race collected by me in Alabama, which ultimately led to the recognition of *simulans*. Mr. BROWN found that males of this race (now known definitely to have been *simulans*), when crossed to mutant races of *melanogaster*, gave only sterile female offspring.

Because of these results observed by Mr. BROWN, I asked Dr. C. W. METZ to send me a number of wild races from Lakeland, Florida, in the spring of 1919. These were tested by crossing to *melanogaster*, and about half of them were found to give unisexual broods. It was noticed that stocks that gave this result looked somewhat different from ordinary *melanogaster*, and had different eggs. Later, Dr. C. B. BRIDGES discovered the striking difference in the male genitalia. Not until many experiments had been made with the Florida race was it noticed that *simulans* also occurs in many other places. I have since shown by crosses, both to the Florida race and to *melanogaster*, that several New York races and one each from New Hampshire and from Minnesota, behave in the same way as does the Florida race. There can now be no question that the male genitalia, by which these later races were first identified, are a certain index as to the genetic behavior.

I have kept mixed stocks of the two species for several months, using the ordinary laboratory technique for keeping stocks of *melanogaster*, and have found that both species may persist together for at least five months. But in such mixed stocks *melanogaster* is usually far more numerous, and the few individuals of *simulans* are almost always small and pale. When the culture becomes old and dry *melanogaster* seems to be unfavorably affected more quickly, so that the percentage of *simulans* rises. Greater resistance to such unfavorable conditions may perhaps account for the fact that *simulans* maintains itself in nature in spite of the fact that, under favorable conditions, it is outdone by *melanogaster*. In the small numbers and small size of the *simulans* specimens from mixed stocks we apparently have the reasons why it was not discovered sooner, for when one selects flies for mating he usually picks out large vigorous ones, and such a procedure would almost always lead to the selection of *melanogaster*. Furthermore, even if *simulans* was occasionally selected it would usually fail to cross, and the absence of offspring would simply be ascribed to sterility.

Drosophila melanogaster ♀ × *D. simulans* ♂

This cross was first made by QUACKENBUSH (1910), to judge from his account. It was also made by Mr. A. M. BROWN, as stated above. I first obtained it in May, 1919, and have since repeated it many times. If the mother is an ordinary XX individual the result is that *only female offspring are produced*. Hundreds of offspring have been obtained from matings of this type, without the production of males.[2] These hybrid females are completely sterile (with *simulans*, *melanogaster*, *obscura*, or hybrid males) and have rudimentary gonads, as QUACKENBUSH first showed.

These females always have the sex-linked characters expected of females from the cross. If the mother carries recessive sex-linked genes that are not present in the father, the corresponding recessive characters are never present in the hybrid daughters. This is true for all the recessive mutant characters tested, 12 in number. If sex-linked recessives are present in the *simulans* father, these also do not appear in the hybrid females. Three such have been tested. It follows that the hybrid females have two X chromosomes, and are not transformed males.

It has been shown by BRIDGES (1916) that females of *melanogaster* that have a Y chromosome (XXY) produce exceptional offspring of two types, due to the production of XX and Y eggs, respectively. The first (XX) type of eggs, if fertilized by Y sperm, produce daughters that get all their sex-linked genes from their mother, and are therefore matroclinous exceptions to the usual behavior of sex-linked characters. The second (Y) type of eggs, if fertilized by X sperm, give rise to sons that get their sex-linked genes from their father, and are thus patroclinous exceptions to the usual behavior of sex-linked characters. In order to further analyze the sex-ratio of the hybrid families, I have crossed XXY *melanogaster* females by *simulans* males. The result has been that *only the regular daughters and the exceptional sons are produced*. The other two classes, regular sons and exceptional daughters, are entirely absent.

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[2] The results to be presented below indicate than an occasional male, due to primary non-disjunction, is to be expected. One male obtained in the cross involving scarlet *simulans* (to be described in part III of these studies) may have been of this nature.

Two experiments of this type have given the same result, and have produced a total of 128 regular females and 59 exceptional males.[3]

Hybrid males produced in this way are sterile, and have rudimentary testes. They are in no way distinguishable from the hybrid males obtained from the reciprocal mating. They have the sex-linked characters of the father, and are identified as non-disjunctional exceptions by this fact.

Drosophila simulans ♀ × *D. melanogaster* ♂

The reciprocal hybrids are more difficult to obtain. They have so far been produced most often from yellow mothers, except in one mating made by Quackenbush. This mating has been made most often by Prof. T. H. Morgan (1920), but I have made it successfully a few times. Usually the *only offspring produced are regular sons*, but Morgan records having sometimes obtained also a few regular daughters, especially as the cultures became old. I have on one occasion used *simulans* females from a stock known to carry extra Y's, and obtained a hybrid female that was undoubtedly a non-disjunctional exception.[4]

As in the case of the reciprocal mating, all these hybrids were completely sterile.

ANALYSIS OF RESULTS

The results of the four types of interspecific matings may be tabulated as follows:

melanogaster ♀ (XX) × *simulans* ♂ = regular ♀♀.
melanogaster ♀ (XXY) × *simulans* ♂ = regular ♀♀ + exceptional ♂♂.
simulans ♀ (XX) × *melanogaster* ♂ = regular ♂♂ + a few regular ♀♀.
simulans ♀ (XXY) × *melanogaster* ♂ = regular ♂♂ + exceptional ♀♀.

Regular females will probably occur in the last mating in small numbers, as in the third mating. It is possible that other classes may appear, but none of them, aside from the exceptional males of the last mating, can survive in nearly as large a percentage of cases as do the types noted.

Analysis of these results indicates that the egg cytoplasm must play a part in determining what types survive. We may represent this cytoplasm by the symbol C, and denote the origin of it and of the chromosomes by sub-letters (m for *melanogaster*, s for *simulans*). The types investigated may then be tabulated as follows:[5]

---

[3] The large number of exceptions is due to the fact that a strain of "high non-disjunction" was used, and does not indicate a higher viability of the exceptional males.

[4] The nature of this female was certain because she had the characteristic appearance of a hybrid, and because she was known to have received a *melanogaster* third chromosome but not a *melanogaster* X chromosome.

♀	♂
$C_mX_mX_s$,—lives	$C_mX_mY_s$,—dies
$C_mX_mX_sY_m$,—lives	$C_mX_mY_mY_s$,—dies
$C_mX_mX_mY_s$,—dies	$C_mX_sY_m$,—lives
$C_sX_mX_s$.—usually dies	$C_sX_sY_m$,—lives
$C_sX_mX_sY_s$,—usually dies	
$C_sX_sX_sY_m$,—lives	

The Y would not be expected to affect the result, and may probably be disregarded. The above formulae then simplify as follows:

♀	♂
$C_mX_mX_s$,—lives	C_mX_m —dies
$C_mX_mX_m$,—dies	C_mX_s,—lives
$C_sX_mX_s$,—usually dies	C_sX_s,—lives
$C_sX_sX_s$,—lives	

This table presents many anomalous points. The difference between the two kinds of regular females (C_m X_mX_s and C_s X_mX_s) can only be due to a difference in the cytoplasm of the mother, since the chromosomes of these two types must be identical.[6]

That hybrid females should develop in *melanogaster* cytoplasm only in case they carry a *simulans* X is surprising. This result must be due in part to the autosomes (or to the *simulans* Y), since C_m X_mX_m, that dies as a hybrid, is the formula of the normal *melanogaster* female. In general, it appears that hybrids develop only if they carry a *simulans* X, but that in the presence of *simulans* cytoplasm a *melanogaster* X usually inhibits development even though a *simulans* X is also present.

FATE OF C_mX_m MALES

It has been assumed in the preceding discussion that the missing classes of hybrids die. It has been shown, by a consideration of the distribution of the sex-linked characters, that they are not changed over into any of the other classes. The possibility of selective fertilization is the only remaining alternative to the assumption that the individuals belonging to the missing classes are formed but die before they reach the adult stage. The evidence now to be presented shows that in the hybrid cultures from *melanogaster* ♀ × *simulans* ♂, many individuals die in the larval stage; and there is every reason to believe that these represent the missing males.

---

[5] The autosomes are alike in all cases (one member of each pair from each parent) and have therefore been left out of the formulae.

[6] It is, of course, probable that the nature of this egg cytoplasm is determined in the first instance by the chromosomes of the female in which the eggs develop.

An eosin vermilion forked *melanogaster* female was mated to a forked *simulans* male. Larvae were observed in about five days, and the pair was removed to a fresh vial. A small amount of food was supplied, and the eggs laid on it were counted and removed, individually, to fresh food. Forty-seven eggs collected in this way gave rise to 14 adults, all of them hybrid forked females as expected. An attempt was made to count the pupa-cases after the adults had all emerged, but this was not found possible. It was clear from the attempt, however, that most of the pupae gave rise to adults, since only a few unhatched puparia could be discovered. Evidently, then, there is heavy mortality in the egg or larval stage. Observation of the isolated eggs indicated that larvae hatched from most of them. A few days after hatching it was noticeable that the larvae were of two sizes;—one type seemed to have grown normally, while the other was much smaller. It had previously been observed that a few larvae were usually present in a hybrid culture at the time adults ceased emerging, and that a number of hybrid larvae died without pupating. These observations indicate that the male larvae grow slowly and never pupate. Their history has not yet been followed in detail, nor has the fate of the other missing classes of hybrids been determined.

SEX-RATIOS OF OTHER SPECIES HYBRIDS

Numerous examples of anomalous sex-ratios among species hybrids are on record. These are evidently due to different causes, as will appear from an examination of a few examples.

When the domestic cow is fertilized by the male bison, the male offspring have a hump, derived from the bison, which is so large that it usually prevents their being born alive. The female hybrids lack this hump, and are born normally (Boyd 1914, etc.). Here, then, an abnormal sex-ratio is brought about at the time of birth by causes external to the hybrid itself,—i.e., by the structure of the mother.

Guyer (1909) has collected data on species hybrids among certain birds, and has shown that there is a decided excess of males in F_1. Although it is possible that some of the individuals were in reality females that had developed male plumage as a result of imperfect development of their ovaries, the evidence indicates a real excess of males. Riddle (1916) has recorded the reverse relation (excess of females) for the cross

Streptopelia risoria × *S. alba* (species of doves) under certain conditions. The cross involves a sex-linked gene, and should give only dark males and white females. Examination of RIDDLE's data for the sex and color of the hybrids shows that, contrary to RIDDLE's own conclusion, the excess of females is not due to "changing over" of males. The female hybrids failed to receive a maternal Z chromosome and would therefore be expected to be females. The data also show that there is not a high enough mortality of embryos to account for the deficiency of males. The only remaining alternative explanation is that, under the conditions of the experiment, the Z chromosome went to a polar body at reduction oftener than it remained in the egg.

GOLDSCHMIDT (1916, 1917, 1919), HARRISON (1919), and others working with Lepidopterous hybrids have often obtained broods that were largely or entirely of one sex. HARRISON has shown that in at least one instance there is not a high enough mortality to account for the results; and both authors have presented evidence indicating strongly that the results are due to a "changing over" of the sex of half the individuals. Crosses in the same groups often produce intersexes, that are more or less intermediate between males and females. These intersexes commonly replace one of the expected sexes, while the other is present in the expected proportions. Intersexuality may be very slight or more and more marked. The unisexual broods evidently represent the final stage where one sex has been completely changed over into the other. The nature of this change is not yet satisfactorily explained; but it is clear that the anomalous sex-ratios are due to causes different from those operating in RIDDLE's doves or in the cow-bison hybrids. The intersexes will be discussed again, in connection with the *simulans* intersexes (part III of these studies).

The *melanogaster-simulans* hybrid sex-ratio evidently represents still a fourth type. It is not due to a changing over of sex, as shown by the distribution of the sex-linked genes. It is not due to a preferential maturation of the egg, since the female Drosophila is not heterozygous for sex. It is not due to external mechanical difficulties, since both types of larvae emerge from the eggs. The cause is an internal one, but acts merely by killing certain classes of larvae.

DESCRIPTION OF HYBRIDS

In general, *melanogaster-simulans* hybrids, of all types that have been observed, are intermediate between the parent species in those characters in which the parents differ. The male genitalia are shown in figure 5.

The width of the cheeks is also intermediate, as is the streak in the dorsal surface of the thorax. Since the hybrid females do not produce eggs, their egg-filaments cannot be examined.

In several respects the hybrids differ from both parents. The complete sterility of both sexes has already been referred to. It is connected with the rudimentary condition of the gonads. The ovaries especially are very minute, and in the testes no sperm have been seen. The hybrids (more especially the females) frequently have fewer dorso-central and scutellar bristles than the number (4 of each sort) that is normal for each parent species. All of these bristles are sometimes missing, though in both pure species individuals with extra bristles are more frequent than those with bristles missing, and I have never seen wild specimens in which more than two were missing. Other bristles on the thorax (noto-pleurals, supra-alars, post-alars) are also often missing in the hybrids but not in the pure species. Another peculiarity that is not infrequent among the

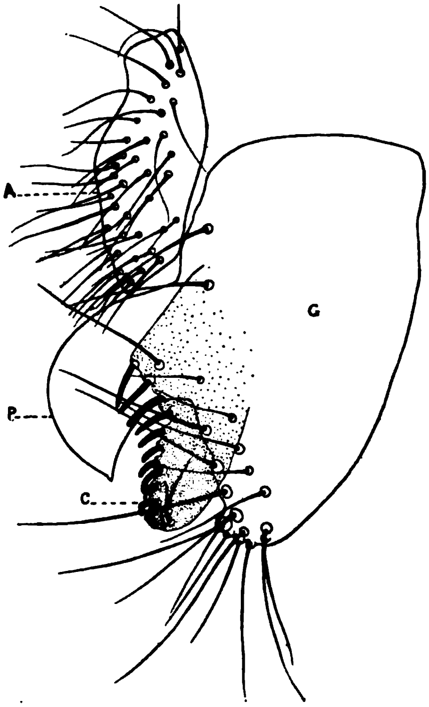

FIGURE 5.—External male genitalia of hybrid male. A, anal plate; C, clasper; G, first genital tergite; P, posterior process of the genital tergite. The upper part of the first genital tergite was broken off in this specimen; this part of the plate is normally shaped as in the two parent species.

hybrids (though less common than the missing bristles) is a broken or missing cross-vein. In the same category belongs abnormally banded abdomen. Both of these characters are very rare in wild individuals of each parent species. None of these characteristics of the hybrids suggests any related species of fly known to me.

These characters that appear in the hybrids but not in either parent species must be due to complemental genes carried by the two species. Since they are evidently dominant genes, it may be surmised that F_2, were it possible to obtain it, would include individuals with numerous other new characters, due to recessive complementary genes.

SEXUAL SELECTION

A small series of observations indicates strongly that females of *melanogaster* or of *simulans* are far more likely to mate with males of their own species than with those of the other species. This is probably the reason why so many cross-matings fail to produce offspring. Both kinds of males will court females of either species, apparently indiscriminately; and that it is the females that are responsible for the small percentage of successful cross-matings is also indicated by the fact that normal *simulans* females have seldom been successfully crossed to *melanogaster* males. QUACKENBUSH used a female that had been subjected to heat, and nearly all later crosses have been from yellow females. I have shown (STURTEVANT 1915) that yellow females of *melanogaster* are more easily mated with by *melanogaster* males than are wild-type females.

Sexual selection, then, is one means whereby the two species are kept from crossing. It would, however, probably be ineffective if fertile hybrids were produced when cross-mating does occur.

Hybrids, of both sexes, have been seen to mate with both parent species. No sufficient data are at hand to show what preferences, if any, are shown by the hybrids, or how readily the parent species will mate with them.

SUMMARY

1. *Drosophila simulans* is a species closely similar to *D. melanogaster* in structure, habits, and chromosome group.

2. The two species may be crossed, though many cross-matings fail, apparently because females do not readily allow males of the other species to mate with them.

3. *Drosophila melanogaster* ♀ × *D. simulans* ♂ produces only female offspring, unless the mother is XXY in constitution. In the latter case

non-disjunction occurs, and regular daughters and exceptional sons are produced.

4. *Drosophila simulans* ♀ × *D. melanogaster* ♂ usually produces only male offspring, but females sometimes come in small numbers. One exceptional female, due to non-disjunction, has also appeared.

5. The regular females from these two matings are of the same chromosome constitution, but in one case they live and in the other they usually fail to emerge. This relation can only be accounted for by supposing that the egg cytoplasm influences the result.

6. Hybrids survive only if they carry a *simulans* X. In the presence of *simulans* egg cytoplasm and a *melanogaster* X, survival is not usual even if a *simulans* X is also present.

7. In the cross of *melanogaster* ♀ × *simulans* ♂, evidence is presented indicating that the regular males die in the larval stage.

8. The sex of the hybrids corresponds in all cases to their chromosome constitution. XX is female, X is male.

9. Hybrids of all classes are completely sterile, and have rudimentary gonads.

10. The hybrids are intermediate between the parent species in all respects in which the latter differ.

11. The hybrids often show some characters (absence of certain bristles, missing or broken cross-vein, abnormally banded abdomen) not present in either pure species.

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SELECTION FOR HIGH AND LOW BRISTLE NUMBER IN THE MUTANT STRAIN "REDUCED"[1]

FERNANDUS PAYNE

Zoological Laboratory, Indiana University, Bloomington, Indiana

[Received April 29, 1920]

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INTRODUCTION

In a former paper (PAYNE 1918) I gave the results of an experiment to increase bristle number on the scutellum of *Drosophila melanogaster*. While plus and minus variations in bristle number may occur in cultures of wild stock, it may be said that four bristles is the normal number in wild flies. In the strain selected for extra bristles, a female with five and a male with four bristles were the starting point. Throughout the experiment which covered 38 generations, flies with the greatest number of extra bristles were used as parents. The mean bristle number was increased to 9.123 in the thirty-fourth generation. The analysis of this line showed that there are at least two and probably more factors concerned in the production of extra-bristle number on the scutellum.

The mutant "reduced" appeared in the sixth generation of the line selected for extra bristles (April 15, 1916). A single male occurred with one bristle on the scutellum. This male was mated to a wild female. In F_1 there were 328 flies, 327 with four bristles and one with five. Five pairs of F_1 flies were mated and gave offspring as follows: 1594 females with four or more bristles, 834 males with four or more bristles, and 804 males with less than four bristles. Of those having less than four bristles, 255 had no bristles, 296, one bristle, 219, two bristles, and 34 three bristles. From these numbers it was evident that the reduced bristle condition behaved as a sex-linked recessive. The character was transferred to the female by mating F_2 reduced males to F_2 females. Of 49 F_2 females tested, 27 were heterozygous for the reduced factor. The offspring of 18 of these heterozygous matings are given in table 1.

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[1] Contribution from the Zoölogical Laboratory of INDIANA UNIVERSITY, No. 173.

TABLE 1

Male "reduced" (1 bristle) × female, wild type (4 bristles).

A. Bristle number of offspring

4 bristles	5 bristles
327	1

B. $F_1 \times F_1$; bristle number of offspring

Females				Males						
4	5	6	7	0	1	2	3	4	5	6
1546	40	6	2	255	296	219	34	826	7	1

C. F_2 reduced male × F_2 heterozygous female; bristle number of offspring

Female						Male						
0	1	2	3	4	5	0	1	2	3	4	5	6
117	166	291	192	1044	3	392	293	181	30	850	2	1

Table 1 gives the results of the cross of the mutant male "reduced" to a wild female; $F_1 \times F_1$; and F_2 heterozygous females × F_2 reduced males. In F_1 there were 327 flies with four bristles and one with five. In F_2 there were 1594 females with four or more bristles, 834 males with four or more bristles, and 804 males with less than four bristles. Practically half of the males then showed the "reduced" character. Note the extra-bristled males and females here, indicating that the reduced male mutant carried factors for extra bristles as well as a factor for the reduced condition. The character reduced is transferred to the female by mating F_2 reduced males to F_2 heterozygous females.

Of the reduced offspring 509 had no bristles, 459 had one, 472 two, and 222 had three bristles.

Here was a mutant character which varied, the number of bristles ranging from zero to three and as we shall see later to four and five. I was working at that time on selection for extra-bristle number and when this variable mutant appeared I planned a second experiment.

THE EXPERIMENT

From the F_3 offspring two lines were started, a minus line by mating parents with no bristles, and a plus line by mating parents with two and three bristles. After the first few generations all parents in the plus line had four bristles. These flies with four bristles were not genetically wild type as they threw the characteristic sex-linked ratios when crossed to the wild. The reduced flies with four bristles could be distinguished readily, however, without the breeding test, as all reduced flies have one or more of the ocellar bristles absent. These two lines, the plus and the minus, have been bred by mating brother and sister for 60 and 65 generations respectively. In the plus line 111,640 flies have been examined and in the minus line 104,362.

The mean bristle number of the flies in the first generation of the plus line was 1.95 (table 2) and the percentage with four bristles was 7.20. For the next two generations these figures were not increased. In fact, they were slightly less in the second and third generations. In the fourth generation, however, the mean rose to 2.08 and the percentage of flies with four bristles to 8.39. The mean in the fifth generation was the same as in the fourth, but from the fifth on it rose gradually, without a break in the increase, to 3.25 in the eighteenth generation. From the eighteenth to the forty-sixth generation the mean fluctuated somewhat, never going below 3.04 nor above 3.35. From the forty-sixth generation there was a rise in the mean to 3.52 in the fifty-fifth generation. From the fifty-fifth to the sixtieth there was a decrease to 3.11 (table 2 and figure 1). If we follow the percentage of flies with four bristles we find a curve similar to that of the mean (tables 2 and 4 and figure 2). From the fifth generation, the percentage rises gradually to 46.90 in the eighteenth generation. From the eighteenth to the forty-sixth generation there is considerable variation, the percentage going as low as 36.20 in the forty-first and as high as 52.94 in the thirty-first. From the forty-sixth to the fifty-fifth generation the percentage rises from 51.06 to 64.25. From the fifty-fifth to the sixtieth there is a decrease to 40.56. The sudden rise in both curves from the forty-sixth to the fifty-fifth generation is somewhat difficult to explain. I was ready to drop the experiment when this sudden rise appeared. The line had remained more or less stationary, except for fluctuations which might be considered environmental, for 28 generations. During this time, more than a year, the line had been subjected to about all the varying environmental conditions which one meets in a laboratory. When the rise came there was

TABLE 2

Summary of plus-selected line.

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 or more bristles	Percent of all offspring with no bristle	Bristle number of offspring												Total
				Female						Male						
				0	1	2	3	4	5	0	1	2	3	4	5	
1	1.95	7.20	8.19	14	36	139	108	48		44	124	156	36	3		708
2	1.88	5.33	11.14	49	231	1081	746	239		469	704	907	214	9		4649
3	1.81	5.57	12.01	54	142	533	326	131		239	417	502	90	5		2439
4	2.08	8.39	4.30	10	47	305	216	105		47	213	323	51	6		1323
5	2.08	7.77	6.32	5	86	469	420	167		135	334	470	121	5		2212
6	2.28	12.60	4.15	2	64	548	616	372		128	409	750	215	22		3126
7	2.36	13.34	2.28	1	30	498	562	361		65	348	759	243	25		2892
8	2.41	15.17	2.42		27	229	302	202		36	153	393	118	23		1483
9	2.54	17.52	1.79		5	117	194	142	1	16	68	254	79	14		890
10	2.83	27.62	0.55		3	70	210	252		6	66	253	170	45		1075
11	2.90	29.57	0.30	1	7	118	355	465	2	5	78	465	357	118		1971
12	2.88	27.51	0.15		3	176	513	603		4	95	662	470	127		2653
13	2.96	30.74	0.43		2	100	361	423		8	62	379	374	148		1857
14	3.01	34.58	0.15		2	46	246	344	3	2	40	297	224	104	2	1310
15	3.03	35.60	0.14		3	43	124	181		1	19	138	126	70		705
16	3.14	41.69	0.31		2	70	169	379	2	4	23	224	254	156		1283
17	3.17	42.00	0.05		3	109	331	580		1	27	298	330	216		1895
18	3.25	46.90	0.00		3	79	257	553			14	259	323	272	1	1761
19	3.21	45.47	0.07		8	152	401	883		2	37	436	513	409		2841
20	3.24	45.67	0.10			37	142	279		1	11	134	171	138		913
21	3.29	47.77	0.09		1	56	176	351	1	1	7	129	204	173		1099
22	3.29	48.23	0.00		2	31	121	247			6	94	141	121		763
23	3.24	47.40	0.00			24	65	153			8	80	97	94		521
24	3.19	42.20	0.00		3	32	105	192			4	101	137	87		661
25	3.12	39.05	0.14		4	76	230	394		2	18	250	275	154		1403
26	3.29	49.19	0.05		5	82	227	587		1	20	219	359	297		1797
27	3.20	43.81	0.06		1	70	206	462	1	1	15	228	288	168		1440
28	3.18	42.72	0.41		1	82	229	393	1	6	23	209	281	226		1451
29	3.30	50.67	0.05		2	78	236	566		1	17	235	309	336		1780
30	3.25	50.64	0.25		3	27	54	112		1	7	48	50	83		385
31	3.35	52.94	0.18		1	22	87	181		1	5	56	76	98		527
32	3.28	52.60	0.00			17	44	119			12	42	49	63		346
33	3.22	46.90	0.54			16	84	185		3	12	83	94	73		550
34	3.05	41.31	0.56		3	20	42	104		2	19	55	65	41		351
35	3.12	42.21	0.32	1	2	47	142	266		2	17	169	158	127		931
36	3.12	40.66	0.00		5	74	192	325			39	191	233	178		1237
37	3.19	43.71	0.19		3	69	234	440		3	33	217	305	231		1535
38	3.20	45.76	0.15		4	31	93	209		1	12	100	111	88		649
39	3.31	50.74	0.19		2	55	214	504		3	21	193	270	277		1539
40	3.23	47.31	0.26		7	53	221	494		4	36	220	263	228		1526
41	3.04	36.20	0.25		2	91	248	411		4	39	306	302	152		1555
42	3.08	37.76	0.07		6	87	196	367		1	28	255	292	158		1390
43	3.35	52.06	0.00		2	33	194	594			12	201	335	250		1621

TABLE 2 (continued)

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 or more bristles	Percent of all offspring with no bristle	Bristle number of offspring												Total
				Female						Male						
				0	1	2	3	4	5	0	1	2	3	4	5	
44	3.16	41.16	0.11		4	85	299	539		2	26	291	355	204		1805
45	3.10	39.73	0.08		11	184	388	631		2	45	368	402	292		2323
46	3.29	51.06	0.00		2	30	85	274			11	118	145	134		799
47	3.42	57.03	0.00			23	91	291			4	86	150	179		824
48	3.35	53.60	0.21		2	31	112	344		2	12	106	172	161		942
49	3.41	55.26	0.00			16	87	228			7	66	130	150		684
50	3.40	56.33	0.20		2	48	192	550		3	15	139	252	290		1491
51	3.47	59.89	0.00		4	57	220	573			11	120	251	417		1653
52	3.51	62.87	0.11			43	259	1029		3	12	233	419	612		2610
53	3.45	60.13	0.25	1		57	328	1277		7	38	307	515	643		3173
54	3.49	63.65	0.17		5	74	376	1585		7	56	334	606	969		4012
55	3.52	64.25	0.23	3	3	112	605	2801		13	65	531	1106	1581		6820
56	3.46	61.31	0.20	1	8	89	448	1882		9	70	468	809	1132		4916
57	3.51	63.23	0.09		5	129	658	2812		7	53	589	1183	1701		7137
58	3.36	54.69	0.07		9	173	589	1971	5	4	98	634	1042	1101		5626
59	3.35	51.68	0.09	1	2	125	302	720		1	19	160	362	320		2012
60	3.11	40.56	0.56	2	6	103	271	489		8	48	281	333	229		1770
Total																111,640

Table 2 gives a summary of the data of the plus-selected line generation by generation. The distribution of the bristles of the males and females is tabulated separately. The mean bristle number, the percentage of all offspring with four or more bristles, and the percentage of all offspring with no bristles are given in each generation. The total number of flies in each generation is also given. A grand total of 111,640 flies were examined during the sixty generations of selection. Note the rise in the mean and in the percentage of flies with four bristles; also the shift in the distribution toward three and four bristles.

nothing unusual about food or room temperature. The rise also lasted for a considerable period, nine generations. The sudden fall in the curve from the fifty-fifth to the sixtieth generation seemed to be due to lack of vitality within the strain. It was impossible to keep the strain going except in mass cultures.[2]

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[2] Since writing the preceding paragraph I have started selection in the plus line again after several months of mass culture. In the first generation the mean rose to 3.51 and the percentage of four-bristled flies to 62.08. In the second generation the mean reached 3.53 and the percentage of four-bristled flies 64.19, practically what they were at the peak of the curve in the fifty-fifth generation. In the third generation there was a slight recession (see table 27). The immediate return of the line to the high point reached in the fifty-fifth generation lends support to the view that the decline in the mean and percentage of four-bristled flies in the last few generations of selection was due to environmental conditions. It further indicates that the rise in the curves from the forty-sixth to the fifty-fifth generation might have been due to a genetic change within the line.

Even if we grant that the rise in the curves from the forty-sixth to the fifty-fifth generation is of no special significance, we must admit that a considerable change within the line has been produced by selection. The mean was increased from 1.95 to 3.01 in the fourteenth generation and it never goes below this in any of the succeeding generations, but does go as high as 3.52 in the fifty-fifth generation. The percentage of flies with four bristles was increased from 7.20 to 35.60 in the fifteenth generation. It never goes below this in any of the succeeding generations, but does go as high as 64.25 in the fifty-fifth generation.

In the minus line the mean bristle number in the first generation was 0.504. There is a marked difference between this mean and that of the first generation in the plus-selected line (1.95). This difference is no doubt due in part to the fact that when F_2 males were mated to F_2 heterozygous females, nearly all the male parents had no bristles. So selection began really in a minus direction when the F_2 parents were mated. The mean in this line was gradually reduced until it reached 0.004 in the seventeenth generation. From this time on to the end of the experiment or to the sixty-fifth generation it remained fairly constant, but fluctuated somewhat, going as high as 0.034 in the twenty-sixth generation and as low as zero in the fortieth (table 3). The percentage of flies with no bristles was gradually increased from 64.61 in the first to 99.52 in the seventeenth generation (table 3 and figure 3). From the seventeenth generation to the end of the experiment this percentage fluctuated slightly, going as low as 96.60 in the twenty-sixth generation and as high as 100 in the fortieth. A glance at table 3 shows that at the beginning of selection in the minus line some of the flies had no bristles, some had one, some two, some three, and a few had four bristles. After the third generation no flies had four bristles; after the seventh generation none had three; after the thirtieth generation only seven flies had two bristles; and after the fifty-sixth no flies had two. It seemed impossible, however, to produce a strain of flies with no bristles. An occasional fly with one bristle appeared in every generation except the fortieth. This generation gave 100 percent zeros.

In the plus-selected line the distribution ranges from zero to four at the beginning of the experiment (table 2). In the first generation 8.19 percent of the flies have no bristles, 22.59 percent have one bristle, 41.66 percent have two bristles, 20.33 percent have three bristles, and 7.20 percent have four bristles. Most of the flies then have one, two, and three bristles with the emphasis on the twos. During the course of selection this distribution is gradually shifted in the direction of four

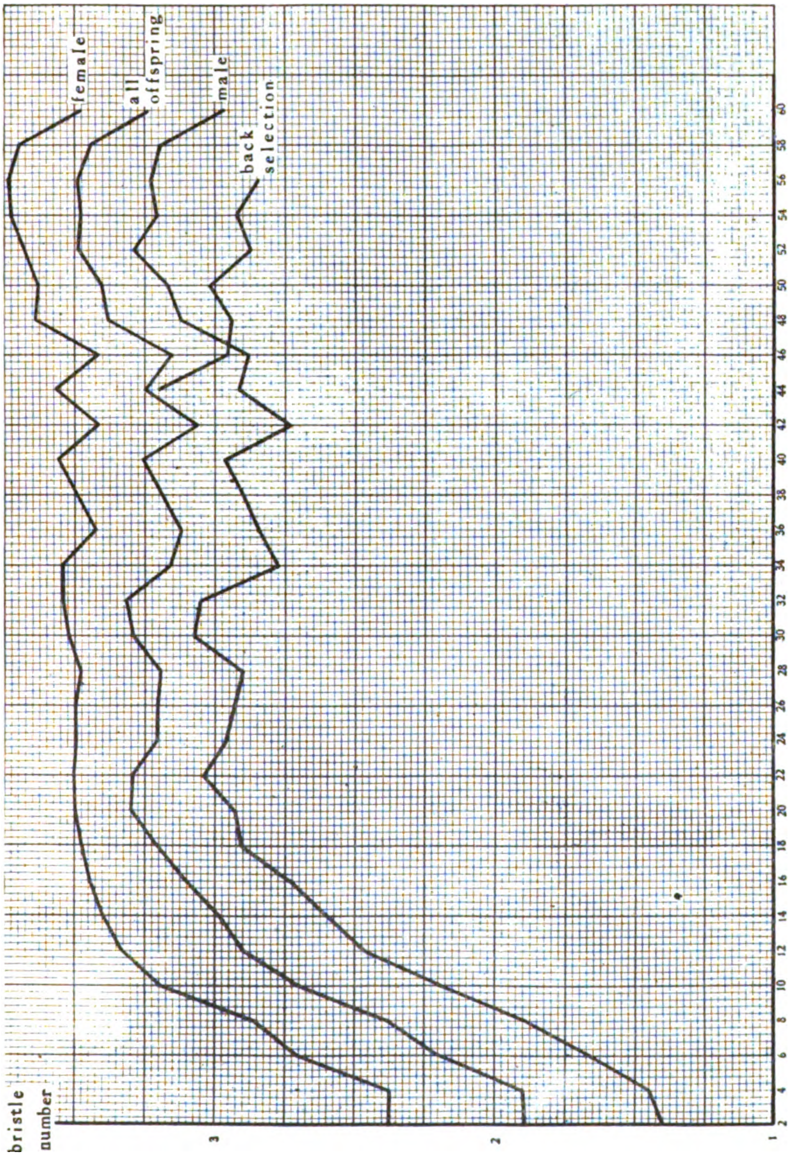

FIGURE 1.—The curves for the mean bristle number in the plus line and the back selection line. The data from which the curves are platted are given in tables 4 and 7.

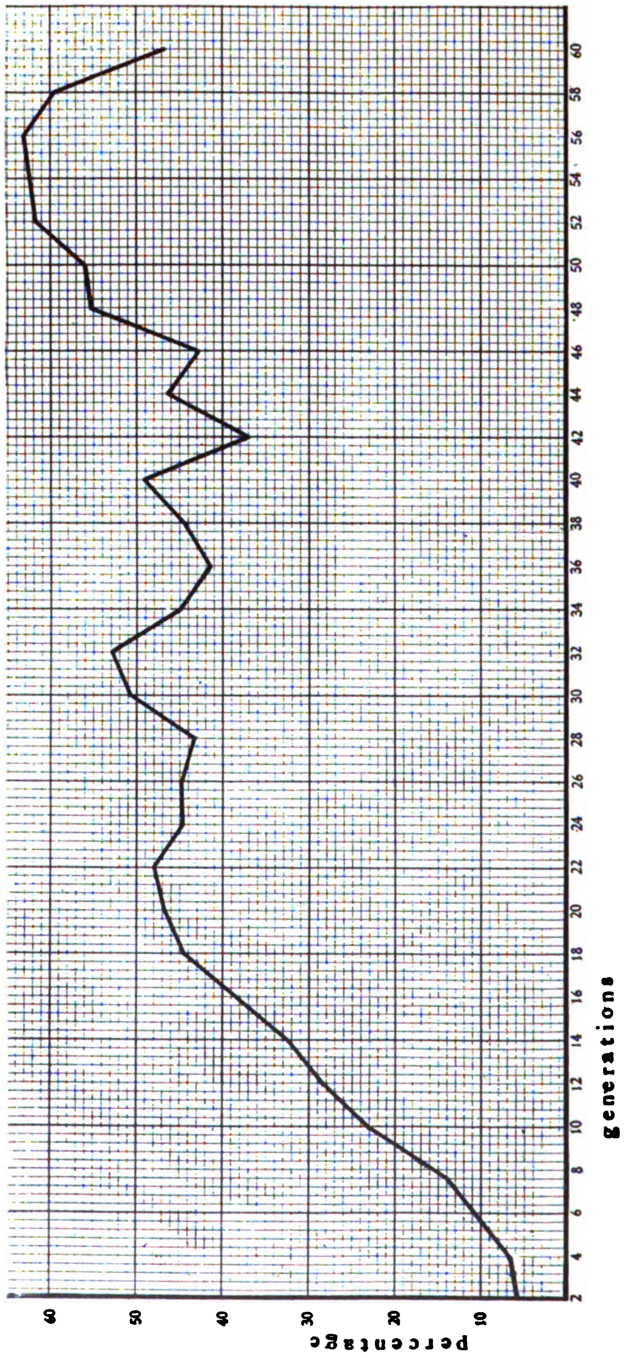

FIGURE 2.—The curve for the percentage of flies with four bristles in the plus line. See table 4 for the data.

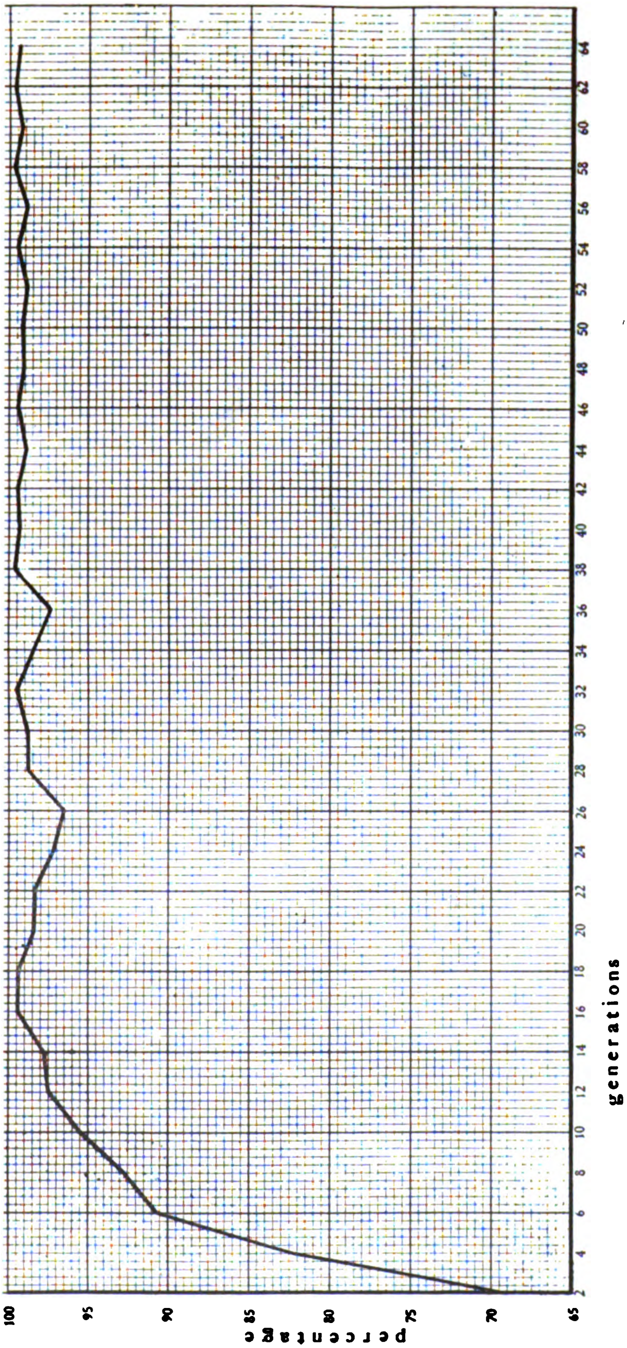

FIGURE 3.—The curve for the percentage of flies with no bristles in the minus line. See table 5 for the data.

TABLE 3

Summary of minus-selected line.

Generation	Mean bristle number of offspring	Percent of offspring with no bristle	Bristle number of offspring									
			Females					Male				
			0	1	2	3	4	0	1	2	3	4
1	0.504	64.61	346	208	134	26	2	581	106	20	4	
2	0.421	70.03	1562	755	368	65	20	2184	311	77	3	4
3	0.293	77.39	888	297	112	10	3	1060	119	28		
4	0.145	88.10	857	146	56			980	46			
5	0.127	89.07	1634	298	60	2		1735	46	7		
6	0.087	92.28	1637	202	37			1724	41	1		
7	0.094	91.85	3050	397	79	3		3379	87	4		
8	0.069	93.78	2533	273	42			2747	35			
9	0.051	95.20	2018	171	17			2113	20			
10	0.044	95.95	1213	76	7			1135	14	2		
11	0.029	97.29	919	41	4			806	3			
12	0.025	97.57	1069	47	3			1064	3			
13	0.024	97.90	759	24	6			737	2			
14	0.023	97.72	820	36	2			857	1			
15	0.006	99.35	612	8				613				
16	0.005	99.45	935	8				894	2			
17	0.004	99.52	1079	9				1024	1			
18	0.009	99.14	1186	14	2			1263	5			
19	0.009	99.06	993	17				1018	2			
20	0.021	97.92	1259	49	1			1336	1			
21	0.014	98.55	1416	39	1			1386	1			
22	0.019	98.01	990	37				1039	4			
23	0.023	97.82	705	26	2			689	3			
24	0.033	96.81	1176	71	3			1252	6			
25	0.032	96.43	837	55	4			920	6			
26	0.034	96.60	666	43	1			757	6			
27	0.017	98.20	324	8				332	4			
28	0.009	99.02	798	15				821	1			
29	0.011	98.92	1050	18	2			1149	4			
30	0.014	98.56	1047	25	1			1013	4			
31	0.007	99.24	665	10				658				
32	0.003	99.69	608	2				695	2			
33	0.011	98.86	466	8				494	3			
34	0.024	97.53	201	10				195				
35	0.011	98.80	40	1				43				
36	0.037	96.69	101	4				104	2	1		
37	0.013	98.64	140	4				152				
38	0.001	99.85	681	2				733				
39	0.009	99.06	497	7				563	3			
40	0.000	100.00	186					194				
41	0.001	99.86	360					366	1			
42	0.014	98.51	118	4				148				
43	0.015	98.45	193	5				190	1			
44	0.009	99.17	274	4	1			327				

TABLE 3 (continued)

Generation	Mean bristle number of offspring	Percent of offspring with no bristle	Bristle number of offspring									
			Females					Male				
			0	1	2	3	4	0	1	2	3	4
45	0.006	99.38	445	6				528				
46	0.006	99.39	956	9				1002	3			
47	0.008	99.16	465	5				484	3			
48	0.010	98.95	930	17				965	3			
49	0.007	99.21	393	3				370	3			
50	0.010	99.10	421	7	1			468				
51	0.019	98.10	366	10				358	4			
52	0.003	99.69	335	2				309				
53	0.005	99.44	279	2				262	1			
54	0.005	99.46	288	3				275				
55	0.005	99.06	496	7	2			462				
56	0.015	98.63	453	10	2			417				
57	0.002	99.73	345	1				417	1			
58	0.004	99.58	442	3				515	1			
59	0.009	99.03	293	6				325				
60	0.008	99.30	266	2				303	2			
61	0.003	99.68	623	4				654				
62	0.004	99.54	566	3				532	2			
63	0.007	99.25	479	6				458	1			
64	0.003	99.66	144					158	1			
65	0.002	99.79	230	1				262				

Table 3 gives a summary of the minus-selected line generation by generation. The bristle counts for the males and females are tabulated separately. The mean bristle number and the percentage of all flies with no bristles are given for each generation. Note the gradual lowering of the mean toward zero and the rise toward 100 in the percentage of flies with no bristles. Note also the shift in the distribution. Toward the end nearly all flies have no bristles. A few have one bristle. In only one generation, the fortieth, was there 100 percent with no bristles.

bristles until in the fifty-fifth generation 64.25 percent of the flies have four bristles, 25.08 percent have three bristles, 9.42 percent have two bristles, 0.99 percent have one and 0.23 percent have no bristles. In the later generations then, most of the flies have three and four bristles.

A comparison of tables 2 and 3 and figures 1 and 2 shows clearly that the minus and plus lines are widely separated at the end of the experiment. They came originally from the same pair of parents. This difference has been produced by selecting parents with few or no bristles on the one hand and parents with the highest number of bristles on the other. The two lines are not only different somatically but they are different germinally. Plus selection has been attempted several times in the minus line without results. Three attempts at minus selection have been made in the plus line without marked results. The data for one of these are given in table 6 and figure 1. The mean drops slightly for the first generation and then remains practically the same

TABLE 4

Summary of the plus-selected line grouped two generations in a group.

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 or more bristles	Percent of all offspring with no bristles	Mean bristle number of females	Percent of females with 4 or more bristles	Mean bristle number of all males	Percent of males with 4 or more bristles	Totals
1, 2	1.89	5.58	10.75	2.38	10.66	1.40	0.45	5357
3, 4	1.90	6.56	9.30	2.37	12.62	1.45	0.58	3762
5, 6	2.20	10.60	5.05	2.70	19.60	1.66	1.04	5338
7, 8	2.38	13.96	2.33	2.86	25.36	1.89	2.22	4375
9, 10	2.70	23.10	1.11	3.19	39.63	2.19	6.07	1965
11, 12	2.89	28.39	0.21	3.33	47.61	2.47	10.26	4624
13, 14	2.98	32.33		3.40	50.42	2.60	15.48	3167
15, 16	3.10	39.63		3.45	57.75	2.73	22.26	1988
17, 18	3.21	44.36		3.48	59.18	2.91	28.08	3656
19, 20	3.30	46.77		3.50	61.09	2.93	29.53	3754
21, 22	3.29	47.95		3.51	60.75	3.04	33.56	1862
23, 24	3.21	44.50		3.49	60.10	2.96	29.76	1182
25, 26	3.21	44.75		3.50	61.12	2.93	28.27	3200
27, 28	3.19	43.27		3.48	59.26	2.90	27.24	2891
29, 30	3.29	50.66		3.52	62.89	3.07	38.54	2165
31, 32	3.32	52.80		3.54	63.69	3.06	40.04	873
33, 34	3.16	44.72		3.54	63.65	2.77	25.50	901
35, 36	3.12	41.32		3.42	56.07	2.84	27.37	2168
37, 38	3.19	44.32		3.49	59.92	2.90	28.97	2184
39, 40	3.27	49.03		3.56	64.38	2.97	33.33	3065
41, 42	3.06	36.94		3.41	55.25	2.73	20.16	2945
43, 44	3.25	46.32		3.57	64.74	2.92	27.08	3426
45, 46	3.15	42.63		3.41	56.38	2.88	28.08	3122
47, 48	3.38	55.20		3.64	71.02	3.12	38.99	1766
49, 50	3.41	56.00		3.63	69.27	3.17	41.82	2175
51, 52	3.49	61.71		3.68	73.31	3.29	49.51	4263
53, 54	3.48	62.26		3.73	77.28	3.21	46.29	7185
55, 56	3.49	63.01		3.74	78.67	3.23	46.90	11736
57, 58	3.44	59.46		3.70	75.38	3.19	43.69	12763
59, 60	3.23	46.48		3.47	59.42	2.96	31.17	3782
Total								111,640

Table 4 gives a summary of the plus-selected line grouped two generations in a group. From the data given in this table the curves (except the one for back selection) in figures 1 and 2 have been made.

TABLE 5

Summary of minus-selected line grouped two generations in a group.

Generation	Mean bristle number of all offspring	Percent of flies with no bristles	Total number of flies
1, 2	0.43	68.96	6776
3, 4	0.22	82.24	4602
5, 6	0.10	90.65	7424
7, 8	0.08	92.71	12629
9, 10	0.04	95.47	6786
11, 12	0.02	97.44	3959
13, 14	0.02	97.81	3244
15, 16	0.005	99.41	3072
17, 18	0.006	99.32	4583
19, 20	0.016	98.41	4680
21, 22	0.016	98.33	4913
23, 24	0.029	98.17	3933
25 26	0.033	96.50	3295
27, 28	0.012	98.78	2303
29, 30	0.013	98.74	4313
31, 32		99.46	2640
33, 34		98.44	1377
35, 36		97.29	296
37, 38		99.64	1712
39, 40		99.31	1450
41, 42		99.49	997
43, 44		98.89	995
45, 46		99.38	2949
47, 48		99.02	2872
49, 50		99.15	1666
51, 52		98.84	1384
53, 54		99.45	1110
55, 56		98.86	1849
57, 58		99.65	1725
59, 60		99.16	1197
61, 62		99.62	2384
63, 64		99.35	1247
Grand total..			104,362

Table 5 gives a summary of the minus-selected line grouped two generations in a group. From these data the curve in figure 3 has been plotted.

for the rest of the thirteen generations. The mean in the first generation was 3.17 and in the thirteenth 3.09. In mass cultures the two lines remain distinct. Each line has been continued in mass culture since the end of the experiment. Small counts were made eight months after the cultures were started. In the minus line 1409 flies were examined. Of these 1401 had no bristles and 8 had one bristle. In the plus line

TABLE 6

Summary of back-selected line.

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 bristles	Bristle number of offspring										Total
			Female					Male					
			0	1	2	3	4	0	1	2	3	4	
1	3.17	41.20		3	26	103	178		7	99	123	75	614
2	2.82	23.95		4	88	189	178	1	24	231	152	39	906
3	2.91	30.40	1	5	68	137	187		18	193	132	55	796
4	2.98	28.73		1	45	128	151		9	146	167	49	696
5	3.04	35.49		2	40	141	213	2	19	153	150	66	786
6	3.00	32.15		1	65	172	245	1	17	210	205	73	989
7	2.91	26.88		2	86	179	169		13	171	139	48	807
8	2.83	25.44		3	97	153	164		14	227	139	52	849
9	2.82	24.16		1	67	114	126	1	22	160	134	33	658
10	3.02	30.64			36	144	165	1	7	130	137	36	656
11	2.85	25.77			65	144	134	1	18	181	115	48	706
12	2.83	24.20		1	43	98	89		24	113	100	32	500
13	3.09	36.65			11	64	103	2	7	62	70	22	341

Table 6 gives a summary of the back-selected line generation by generation. It gives the distribution of the bristles in the males and females, the mean bristle number, and the percentage of all offspring with four bristles in each generation. Note that the mean was 3.17 in the first generation and 3.09 in the thirteenth.

TABLE 7

Back-selected line summarized two generations in a group.

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 bristles	Total
1, 2	2.96	30.92	1520
3, 4	2.94	29.62	1492
5, 6	3.02	33.63	1775
7, 8	2.87	26.14	1656
9, 10	2.92	30.64	656
11, 12	2.84	25.12	500

Table 7 gives the data for the back-selected line grouped two generations in a group. From these data the curve for the back-selection line in figure 1 is plotted.

544 flies were examined. Of these 277 had four bristles, 156 three bristles, 96 two bristles, 14 one bristle, and one no bristles. The mean of these is 3.27. The mean of the sixtieth generation at the end of selection was 3.11. Both of these counts, then, show that the two lines have remained for eight months practically the same as they were at the end of selection. They have been not only separated by selection but they have become fixed by selection.

THE ANALYSIS OF THE DIFFERENCES

What is this germinal difference and how has selection brought it about? In an attempt to answer these questions I have made use of the phenomena of linkage and crossing over and have also made other crosses to bring out the genetic differences.

Before taking up the analysis of the germinal differences between the two lines a few words should be said about the effect of the environment upon bristle number. My own observations during selection had led me to think that temperature was one factor which caused considerable variation. Following up this notion one of my students, Mr. ALBERT FROEMMING, made some experiments in which he demonstrated that low temperature increases bristle number in both the plus and minus lines. His experiments were made after about one year of selection.

In the experiments with the plus line he mated females with three bristles to males with two bristles. First he placed the parents in an incubator with the temperature varying from 23° to 26°C. Here the bottles were allowed to remain until the flies had completed their life cycle, which in this case was about ten days. He examined 7668 offspring. They showed a mean bristle number of 2.03. A second experiment was made in a cave room where the temperature varied from 10 to 13°C. At this temperature the adults would not deposit their eggs. So they were kept at room temperature until larvae appeared in the bottles. The bottles were then transferred to the cold room. They were kept here until the larvae pupated. Pupation would occur in the cold but the flies would not emerge. Hence after pupation the bottles were again transferred to room temperature. Three thousand two hundred twenty-five flies gave a mean bristle number of 2.40. The life cycle in this case was about thirty days. The flies bred in the cold room have a higher mean bristle number than those bred in the incubator.

Another experiment and even more convincing than the first two, was carried out by using the same parents under the different temperatures. He permitted the bottles to remain at room temperature until larvae appeared. The parents were transferred to new bottles and the bottles with larvae transferred to the cave room. The new bottles with the parents were put into the incubator. The mean bristle number of the 2047 flies reared at 10° to 13°C was 2.34; that of the 2929 flies reared in the incubator at 23° to 26°C was 2.14. These experiments leave no doubt, I think, that low temperature increases bristle number in the plus-selected line. They also demonstrate that the effect is produced during larval life.

In the minus-selected line similar experiments were made. One will suffice to demonstrate the effect of the lower temperature in increasing bristle number. Flies with no bristles were used as parents. They were placed in bottles and permitted to remain until they had deposited eggs. The parents were removed to bottles with fresh food and placed in the incubator. The bottles with eggs and larvae were placed in the cold room. Those reared in the incubator (804) gave 96.64 percent with no bristles; those reared in the cave room (542) gave only 79.52 percent with no bristles. In the minus-selected line, then, also, the lower temperature increases bristle number.

These conclusions reached about three years ago agree with the recent work of SEYSTER (1919) on the effect of temperature on facet number in the bar-eyed mutant.

A study of tables 2 and 3 shows clearly that at the end of selection the plus line is much more variable than the minus line. In the minus line only a very few flies have one bristle. The rest have none. In the plus line most of the flies have three and four bristles but flies also appear with two, one, and no bristles. The figures and tables also show that the females have more bristles than the males. This is brought out in figure 1 and table 4 for the plus line. In the early generations the difference is greater than later when the line has become somewhat stable. It approaches and even reaches one in the ninth and tenth generations. After the sixteenth the difference is about one-half and remains fairly constant. In the line selected for extra bristles (PAYNE 1918) a similar difference between males and females was present. The analysis showed that a sex-linked factor was responsible for the difference. The plus line in the present experiment has been tested for such a factor. The case here is slightly different, for the reduced condition itself is due to a sex-linked factor. Could there be another sex-linked factor, which modifies bristle number, in addition to the primary one? The analysis shows the presence of such a factor. The test was made by crossing the plus line to eosin miniature and then mating the F_1 female to an eosin miniature male. Table 8 gives the results. Only males are included in the table as all females in this type of cross are wild type with respect to bristle number. Reduced and eosin miniature are the non-crossover classes. Of the crossover classes miniature reduced and eosin are the largest. Reduced and eosin are found together in only a small percentage of the crossovers. These facts demonstrate that eosin and reduced lie very close together. If there are factors in the X chromosome, other than reduced, which modify bristle number, a comparison of the crossover class, miniature reduced, with the non-crossover class, reduced, ought to bring this out. If there are no other factors, the miniature reduced flies should show approximately the same bristle number and distribution as the reduced. On the other hand, if there are additional factors, and if they cross over in the F_1 female, then the two classes should differ with respect to bristle number and distribution. A comparison of the reduced and the miniature reduced flies show very clearly a difference.

TABLE 8

F_1 ♀ (*from plus line* ♀ × *eosin miniature* ♂) × *eosin miniature* ♂.

Type	Number of bristles	Number of male off-spring	Percentage	Mean
Wild	4	55		
Reduced	3	78	5.78	1.022
	2	371	27.54	
	1	402	29.84	
	0	496	36.82	
Miniature	4	11		
Miniature reduced	3	20	2.64	0.748
	2	147	19.47	
	1	211	27.94	
	0	377	49.93	
Eosin miniature	4	1324		
Eosin miniature reduced	3	1		
	2	5		
	1	10		
	0	13		
Eosin	4	682		
Eosin reduced	3	2		
	2	2		
	1	0		
	0	2		

Table 8 gives a summary of the results when an F_1 female produced by mating the plus line to eosin miniature, is back-crossed to an eosin miniature male. The data are for the males only as none of the females show the character "reduced." It gives the number of flies and their bristle number in each class. Reduced and eosin miniature are the non-crossover classes. Of the crossover classes, miniature reduced and eosin are by far the largest. This table affords a comparison of the non-crossover class, reduced, with the crossover class, miniature reduced. The two are different, demonstrating the presence of a plus-modifying factor in the X chromosome near miniature.

TABLE 9

F_1 ♀ *(from plus line* × *eosin ruby forked)* × *eosin ruby forked* ♂.

Type	Number of bristles	Number of male offspring	Percentage	Mean
Wild	4	44		
Reduced	3	63	13.54	1.301
	2	135	29.03	
	1	146	31.39	
	0	121	26.02	
Forked	4	23		
Forked reduced	3	25	7.28	1.088
	2	82	23.90	
	1	107	31.19	
	0	129	37.60	
Eosin ruby forked	4	499		
Eosin ruby forked reduced	3	3		
	2	4		
	1	1		
	0	4		
Eosin ruby	4	279		
Eosin	4	33		
Eosin forked	4	4		
Ruby forked	4	1		
Ruby forked reduced	3	2		
	2	5		
	1	11		
	0	9		
Ruby	2	6		
	1	1		
	0	3		
Eosin ruby reduced	2	1		
	0	2		

Table 9 shows the results of back-crossing an F_1 female, produced by crossing the plus line to eosin ruby forked, to an eosin ruby forked male. The reduced and eosin ruby forked flies are the non-crossovers. The forked reduced and eosin ruby are the principal crossover classes. A comparison of the non-crossover class, reduced, with the crossover class, forked reduced, shows the reduced flies to have a higher mean bristle number, 1.301 as compared to 1.008 for the forked reduced. Of the reduced flies only 26.02 percent have no bristles while 37.60 percent of the forked reduced have no bristles.

The mean of the reduced flies is 1.022 while in the miniature reduced flies it is 0.748. The distribution of the bristles differs also. Of the reduced flies 36.82 percent have no bristles; of the miniature reduced flies 49.93 percent have no bristles. It would seem then that in addition to the factor for reduced we have an additional modifying factor near miniature which does not pass into the miniature reduced crossover class. Hence this class has fewer bristles than the non-crossover class, reduced.

The same fact is shown, but less clearly, by mating the plus line to eosin ruby forked and back-crossing the F_1 females to eosin ruby forked males (table 9). A comparison of the non-crossover class, reduced, with the crossover class, forked reduced, shows that the reduced flies have a higher mean bristle number (1.301) than the forked reduced (1.008). There are also fewer flies with no bristles, 26.02 percent as compared with 37.60 percent in the forked reduced. The fact that the differences here are not so marked as in the eosin miniature cross is no doubt due to the fact that the second factor lies near miniature and in many of the crossovers both bristle factors cross over. This would raise the bristle number of the forked reduced flies above that of the miniature reduced.

Attempts were also made to locate factors outside the X chromosome. First the plus line was mated to black pink bent stock and the F_1 females back-crossed to black pink bent males. The data for the males which show the reduced character are given in table 10. So far as I can see, though, it gives no certain clew. One fact stands out very clearly, however, and that is that the bent reduced have practically the same mean bristle number and the same distribution as the reduced flies. The mean of the bent reduced is 1.20 and of the reduced flies it is 1.19. In contrast with this the black reduced, and pink reduced flies have a much lower mean and a much higher percentage of the flies have no bristles. The mean for the black reduced flies is 0.35 and for the pink reduced 0.72. This indicates the possibility of plus modifying factors in the second and third chromosomes. The plus line was also crossed to black, to spineless, to sooty and to eyeless. In each case the F_1 female was back-crossed to black, to spineless, to sooty, and to eyeless males, respectively (table 11). These crosses brought out the same thing as the cross to black pink bent. The black reduced, spineless reduced, and sooty reduced flies had a lower mean bristle number than the reduced. The eyeless reduced flies, as in the case of the bent reduced, had practically the same mean and distribution as the reduced flies.

TABLE 10

F_1 ♀ (from plus line ♀ × black pink bent ♂) × black pink bent ♂.

Type	Number of bristles	Number of male offspring	Percentage	Mean
Reduced	3	28	11.81	1.19
	2	71	29.95	
	1	57	24.05	
	0	81	34.17	
Black reduced	3	3	1.57	0.35
	2	13	6.80	
	1	33	17.27	
	0	142	74.34	
Pink reduced	3	15	7.10	0.72
	2	28	13.27	
	1	51	24.17	
	0	117	55.45	
Bent reduced	3	9	9.78	1.20
	2	28	30.43	
	1	28	30.43	
	0	27	29.34	
Black pink reduced	3	0	0.00	
	2	7	4.45	
	1	12	7.64	
	0	138	87.89	
Black bent reduced	3	0	00.00	
	2	13	13.54	
	1	26	27.08	
	0	57	59.37	
Black pink bent reduced	3	0	00.00	
	2	3	4.91	
	1	11	18.03	
	0	47	77.04	
Pink bent reduced	3	8	8.78	
	2	21	23.07	
	1	18	19.77	
	0	44	48.35	

Table 10 gives the results of back-crossing an F_1 female (produced by crossing a plus line female to black pink bent male) to a black pink bent male. Only the males showing the character reduced are given in the table as all others are wild type with respect to bristle number. The factors for black, pink and bent are located in the second, third and fourth chromosomes, respectively. A comparison of the reduced flies with black reduced, pink reduced and bent reduced, shows that the mean and distribution of the bristles in reduced and bent reduced are very similar. On the other hand the mean in the black and pink flies is very much less and the distribution is shifted toward the zeros.

TABLE II

A. *F*₁ ♀ (*from plus line* × *black*) × *black* ♂.

Reduced			Black reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	68	15.17	3	11	2.55
2	117	26.11	2	45	10.44
1	131	29.24	1	93	21.57
0	132	29.46	0	282	65.42

B. *F*₁ ♀ (*from plus line* × *spineless*) × *spineless* ♂.

Reduced			Spineless reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	17	3.60	3	4	0.94
2	43	9.12	2	4	0.94
1	68	14.43	1	16	3.78
0	343	72.82	0	399	94.32

C. *F*₁ ♀ (*from plus line* × *sooty*) × *sooty* ♂.

Reduced			Sooty reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	20	5.31	3	8	2.65
2	54	14.36	2	11	3.65
1	94	25.00	1	35	11.62
0	208	55.31	0	247	82.05

D. *F*₁ ♀ (*from plus line* × *eyeless*) × *eyeless* ♂.

Reduced			Eyeless reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	56	11.02	3	49	12.37
2	98	19.29	2	118	27.79
1	140	27.55	1	107	27.02
0	214	42.12	0	122	30.80

TABLE 11 (continued)

E. F_1 ♀ (*from plus line* × *wild*) × *wild* ♂.

Reduced		
Bristle number	Number of flies	Percent
4	18	3.73
3	49	10.16
2	105	21.78
1	116	24.06
0	194	40.24

Table 11 gives a series of crosses of the plus line to black, to spineless, to sooty, to eyeless and to the wild, and then in each case back-crossing the F_1 female to the black male, the spineless male, the sooty male, the eyeless male and the wild-type male, respectively. The table gives only the distribution of the bristles in the males which show the character reduced. The factor for black is in the second chromosome, those for spineless and sooty in the third, and that for eyeless in the fourth. These crosses are similar to the one made to black pink bent, and the results are also very similar, i.e., the eyeless reduced flies are similar in mean and distribution of bristles to the reduced, while the black, spineless and sooty have a lower mean and the distribution is shifted toward the zeros.

In an attempt to determine the presence or absence of factors in the third chromosome, the plus line was crossed to sepia spineless kidney sooty rough, the factors of which lie in the third chromosome. The F_1 females were back-crossed to sepia spineless kidney sooty rough males. Four groups of the males, because only the males show the reduced character, have been recognized. These are: First, reduced; second, sepia spineless kidney sooty rough reduced; third, sepia reduced and sepia reduced in combination with any other factor or factors except rough; and fourth, rough reduced and rough reduced in combination with any other factor or factors except sepia (table 12). A study of this table shows that the means and the distributions of the bristles of the reduced and the rough reduced flies are very similar. The mean of the reduced is 0.995; that of the rough reduced 0.946. Of the reduced flies 38.28 percent have no bristles; of the rough reduced 38.39 percent have no bristles. On the other hand the sepia reduced and the sepia spineless kidney sooty rough reduced males have a similar mean and a similar distribution of bristles. The mean of the former is 0.680; that of the latter 0.642. The percentage of flies with no bristles is 58.82 in the sepia reduced and 58.89 in the sepia spineless kidney sooty rough reduced. Table 12 is a compilation of two separate crosses made at different times. Each by itself showed practically the same result as the sum of the two.

This shows very clearly, I think, the presence of a factor in the third chromosome near the sepia end. Otherwise why the difference in these two types of crossovers? No further study was made of the second chromosome because of lack of suitable material for analysis.

TABLE 12

F_1 ♀ (from plus line × sepia spineless kidney sooty rough) × sepia spineless kidney sooty rough ♂.

Type	Number of bristles	Number of male offspring	Percentage	Mean
Reduced	3	16	7.20	0.995
	2	52	23.42	
	1	69	31.08	
	0	85	38.28	
Sepia reduced and sepia reduced in combination with any other factor or factors except rough	3	8	6.72	0.680
	2	16	13.45	
	1	25	21.00	
	0	70	58.82	
Rough reduced and rough reduced in combination with any other factor or factors except sepia	3	9	8.03	0.946
	2	19	16.96	
	1	41	36.60	
	0	43	38.39	
Sepia spineless kidney sooty rough reduced	3	8	4.39	0.642
	2	28	15.38	
	1	37	20.32	
	0	109	59.89	

Table 12 gives the results of back-crossing F_1 females (produced by crossing the plus line to sepia spineless kidney sooty rough stock) to sepia spineless kidney sooty rough males. The factors for these characters lie in the third chromosome. Only males showing the character reduced are given in the table. The reduced and the sepia spineless kidney sooty rough reduced are the non-crossover classes. Only two classes of crossovers are recognized. These are, first, sepia reduced and sepia reduced in combination with any other factor or factors except rough; and second, rough reduced and rough reduced in combination with any other factor or factors except sepia. The mean and bristle distribution in the reduced and rough reduced class of crossovers are practically the same. Also the mean and distribution of the bristles in the sepia spineless kidney sooty rough reduced and in the sepia reduced class of crossovers are about the same, but they are different from the other two.

We are forced then to conclude that in the plus line, at least three factors are present which modify bristle number, one primary factor which we have called "reduced," and which keeps the bristle number below that of wild flies, and two modifying factors which increase bristle number up to the same as or near that of wild flies.

In the analysis of the minus-selected line, the line was crossed to eosin miniature and the F_1 females back-crossed to eosin miniature males. A comparison of the reduced and the miniature reduced classes shows them practically the same with respect to bristle number. The mean of the reduced flies is 0.071 and of the miniature reduced flies 0.061. The percentage of flies with no bristles among the reduced is 93.04 and among the miniature reduced it is 94.56 (table 13). Hence we are forced to conclude that in the minus line the modifying factor found in the X chromosome of the plus line is not present.

TABLE 13

F_1 ♀ (from minus line ♀ × eosin miniature ♂) × eosin miniature ♂.

Type	Number of bristles	Number of male offspring	Percentage
Reduced	2	2	0.24
	1	55	6.70
	0	763	93.04
Miniature reduced	2	3	0.74
	1	19	4.69
	0	383	94.56
Miniature	4	2	
Eosin reduced	0	3	
Eosin	4	359	
Eosin miniature	4	767	
Eosin miniature reduced	1	1	
	0	23	
Wild	4	27	

Table 13 gives the results of back-crossing an F_1 female (produced by crossing the minus-selected line to eosin miniature) to an eosin miniature male. Reduced and eosin miniature are the non-crossover classes. Miniature reduced and eosin are the principal crossover classes. A comparison of the non-crossover class, reduced, with the crossover class, miniature reduced, shows the two very similar.

The minus line was also crossed to black pink bent and the F_1 females back-crossed to black pink bent males (table 14). The mean and the distribution of the bristles were very similar in the reduced, black, reduced, pink reduced, and bent reduced males. Hence we see no indication of factors outside the X chromosome.

TABLE 14

F_1 ♀ (from minus line ♀ × black pink bent ♂) × black pink bent ♂.

Type	Bristle number	Number of male offspring	Percentage
Reduced	3	0	00.00
	2	3	1.33
	1	14	6.22
	0	208	92.44
Black	3	0	00.00
	2	0	00.00
	1	4	2.11
	0	185	97.88
Pink	3	0	00.00
	2	0	00.00
	1	33	15.06
	0	186	84.93
Bent	3	0	00.00
	2	0	00.00
	1	2	1.68
	0	117	98.31
Black pink	3	0	00.00
	2	0	00.00
	1	2	1.45
	0	135	98.54
Black bent	3	0	00.00
	2	0	00.00
	1	3	4.00
	0	72	96.00
Black pink bent	3	0	00.00
	2	2	2.89
	1	2	2.89
	0	65	94.20
Pink bent	3	0	00.00
	2	0	00.00
	1	11	10.09
	0	98	89.90

Table 14 gives the results of crossing F_1 females (produced by mating the minus line to black pink bent) to black pink bent males. Only the males showing the character reduced are tabulated as all other flies are wild type with respect to bristle number. The percentage of zero flies and the distribution of the bristles is practically the same in each class.

We must conclude then that in the minus line only a single factor is present and that this acts as an inhibitor which prevents the appearance of the normal bristle number.

In a further attempt to test the genetic differences between the plus and minus lines, the two lines were crossed out to the wild, with each other, and also to the line selected for extra bristles. Can the results of these crosses be explained on the basis of the conclusion that in the minus line there is a single inhibiting factor present and in the plus line this same inhibitor and two or more plus factors? In fact if the conclusions are correct they must explain the results.

Let us consider the plus line first. When the plus-line male is crossed to a wild female and the F_1 flies inbred, one-half of the F_2 males are reduced. Of these extracted F_2 males 15.57 percent have four bristles, 24.84 percent have three bristles, 32.21 percent have two bristles, 20.42 percent have one bristle, and 6.94 percent have no bristles (table 15). The males of the parental generations showed 46.71 percent with four bristles, 32.34 percent with three bristles, 18.35 percent with two bristles, 2.22 percent with one bristle, and 0.35 percent with no bristles. The mean of the males in the parental generations was 3.22 while in the extracted F_2 males it was 2.21. This is a considerable regression toward the unselected line. The ninth and tenth generations of selection showed a mean of 2.19 for the males and the thirteenth and fourteenth generations showed 15.48 percent with four bristles (table 4). A certain amount of regression should be expected, however, if there are three factors for bristle number in the plus line, and if they become separated, as they would, in such an out-cross.

TABLE 15

F_1 × F_1 (from plus line male × wild female).

	Female	Male, wild type	Male, reduced				
Bristle number	4	4	0	1	2	3	4
Frequency	983	482	33	97	153	118	74
Percent	100.00	100.0	6.94	20.42	32.21	24.84	15.57

Table 15 gives the results of inbreeding the F_1 flies produced by crossing the plus-line male to a wild female. About one-half of the males show the character reduced. Note the distribution of the bristles in the reduced males.

The plus-line female was crossed to the wild and the F_1 males compared with the F_2 extracted males. Table 16 gives the results. Of these males 17.65 percent have four bristles, 26.23 percent have three bristles, 40.10 percent have two bristles, 12.50 percent have one bristle, and 3.50 percent have no bristles. The mean is 2.42. Here also is a considerable regression. Perhaps more than we should expect. The difference between these males and the F_2 extracted males is not very great. The F_1 males get their X chromosome from the plus-line female and hence they have the inhibitor and one plus modifier. The second plus modifier is also present in a heterozygous condition. The F_2 extracted males get their X chromosome from the plus-line male. Some of these males would be homozygous for the factor in the third chromosome, some would be heterozygous and some would not have it at all. The inhibitor and one plus modifier are sex-linked. They may become separated by crossing over in the F_1 female. If separated, the bristle number would be decreased, as a fly without the inhibitor is a wild-type fly. Hence, upon analysis the difference between these groups of males should not be so great.

TABLE 16

Plus-line female × wild male.

	Females	Males, reduced				
Bristle number	All wild type	0	1	2	3	4
Frequency		51	182	584	382	257
Percent	100.00	3.50	12.50	40.10	26.23	17.65

Table 16 gives the results of crossing plus-line females to wild males. All the males are reduced as they get their X chromosomes from the plus-line female. Note the distribution of the bristles.

The one puzzling result is the cross of the plus-line female to the minus-line male. If there is a single factor in the X chromosome of the minus line, the F_1 males from this cross might be expected to have as many bristles as the F_1 males when the plus-line female is crossed to a wild male. They do not, however. Table 17 shows only 3.32 percent of them have four bristles, 9.20 percent have three bristles, and 29.71 percent have two bristles, while 33.03 percent have one bristle and 24.72 percent have no bristles. Compare these percentages with those in table 16 where 17.65 percent of the males have four bristles. Why this difference when in both cases the F_1 male gets his X chromosome from the plus-line female? The difference must be due to factors outside the X chromosome. While at first glance these results seem difficult to understand, yet perhaps they should not be counted as opposing our conclusions when we recall that we know nothing of the factor or factors which keep the number of bristles in wild flies practically constant. Certainly the normal number cannot be due to a single factor. In fact, I am convinced that several bristle factors are present in wild flies.

Wild flies differ genetically with respect to extra-bristle factors. During my selection experiments several strains have been tested. In one strain (PAYNE 1918) the bristle number was increased until the mean was nine plus. All wild strains which have been examined have a few flies with one extra bristle. Selection has been tried in two strains without results. At the present time I have a strain which has been selected for extra bristles for six generations. The mean in the fifth generation is 5.17 and 75.60 percent of the flies have extra bristles (table 18). Yet in this strain no fly has had more than seven bristles and only a few have that number. Nearly all of the extra-bristled flies have one or two extra bristles. As yet I am not absolutely sure, but I feel convinced that, unless some further genetic change occurs in the strain, I shall never be able to increase the mean much beyond what it is at the present time. I may be able to produce a strain in which 100 percent of the flies have extra bristles, but the number of bristles will not go beyond seven. It seems to me then that in the wild fly there may be plus modifiers which have been eliminated in the minus line by selection.

TABLE 17

Plus-line female × minus-line male.

	Female bristle number					Male bristle number				
	0	1	2	3	4	0	1	2	3	4
	93	207	385	236	153	223	298	268	83	30
Percent						24.72	33.03	29.71	9.20	3.32

Table 17 gives the results of crossing the plus-line female to the minus-line male. Note drop in the percentage of flies with four bristles and the increase in the other groups, when compared with table 16.

We meet with no difficulties when similar crosses are made with the minus line. When the minus-line female is crossed to a wild male 78.56 percent of the F_1 males have no bristles, 18.58 percent have one bristle, and 2.85 percent have two bristles (table 19). Here as in the plus line

TABLE 18

Summary of selection for extra bristles in a second wild strain.

Generation	Mean bristle number of parents	Bristle number of offspring							Total	Mean bristle number of offspring	Percent with extra bristles	Percent with seven bristles
		Female				Male						
		4	5	6	7	4	5	6				
1	4.50	268	31	3		268	3		573	4.06	6.45	—
2	4.70	622	138	70	1	660	48	9	1548	4.22	17.82	—
3	5.61	283	149	80	5	372	62	6	957	4.41	31.55	—
4	5.80	50	76	62	5	146	52	13	405	4.72	51.48	1.23
5	6.00	31	68	143	6	80	92	35	455	5.17	75.60	1.31
6	6.00	50	107	159	7	126	112	64	625	5.09	71.84	1.12

Table 18 gives a summary of a second line selected for extra bristles. The starting point was three females, each with one extra bristle, and three wild-type males. The three pairs were from the same stock culture which had been inbred in the laboratory for two or three months. The mean bristle number of the offspring gradually rises from 4.06 in the first generation to 5.17 in the fifth. In the sixth there is a slight decrease to 5.09. No flies have appeared with more than seven bristles, and only a few with that number. It is doubtful whether selection will carry the change much further.

TABLE 19

Minus-line female × *wild male.*

Females	Male bristle number				
4	0	1	2	3	4
All wild type	689	163	25		
Percent................	78.56	18.58	2.85	0.00	0.00

Table 19 gives the results of crossing minus-line females to wild males. All the males are reduced as they get their X chromosomes from the minus-line female. Note the distribution of bristles.

TABLE 20

$F_1 \times F_1$ *(from wild female* × *minus-line male).*

Female	Male, wild type	Male, reduced				
4	4	0	1	2	3	4
557	849	632	160	57	6	2
Percent............................		73.74	18.66	6.65	0.70	0.23

Table 20 gives the results of inbreeding the F_1 flies produced by crossing the minus-line males to wild females. One-half of the F_2 males show the character reduced. Note the distribution of the bristles in these reduced males.

is a regression toward the unselected line. It indicates, as suggested, that the wild fly has more than one bristle factor. In this case it would seem that plus modifiers in the wild fly cause the change. If the minus-line male is crossed to the wild and the F_1 individuals inbred, the F_2 reduced males show about the same regression as the F_1 males show when the minus-line female is crossed to the wild (table 20). The cross of the minus-line female to the plus-line male brings out an interesting fact. Table 21 shows that only 51.67 percent of the F_1 males have no bristles.

TABLE 21

Minus-line female × plus-line male.

	Female				Male				
0	1	2	3	4	0	1	2	3	4
90	185	283	174	68	416	226	136	23	4
Percent					51.67	28.07	16.89	2.85	0.49

Table 21 gives the results of crossing the minus-line female to the plus-line male. Note particularly the drop in the percentage of males with no bristles. The drop is much greater than when the minus-line female is crossed to the wild.

TABLE 22

Wild ♀ × plus line ♂.

Generation	Mean bristle number of parents	Bristle number of offspring													Totals	Number with extra bristles	Mean bristle number of offspring	Percentage of flies with extra bristles
		Females					Males			Males, reduced								
		4	5	6	7	8	4	5	6	0	1	2	3	4				
F_1	4.00	221	5				218	4							448	9	4.02	2.00
$F_1 \times F_1$	4.37	352	51	17			190	10	3	17	30	56	46	41	623	81	4.16	13.00
$F_2 \times F_2$	5.06	441	102	20	1		566	28	1						1159	152	4.15	13.11
$F_3 \times F_3$	5.43	152	98	34	3		231	45	4						567	184	4.40	32.45
$F_4 \times F_4$	5.68	195	159	61	12	7	351	58	4						847	301	4.48	35.53
$F_5 \times F_5$	6.50	109	94	76	2		180	78	34						573	284	4.69	49.56
$F_6 \times F_6$	5.92	37	66	62	7		88	41	13						315	190	4.89	60.31

Table 22 gives the results of crossing the plus-line male to a wild female and inbreeding for six generations the flies with extra bristles. The mean in F_1 is 4.02 and the percent of flies with extra bristles is 2. These figures are gradually increased in later generations of inbreeding until in F_7 the mean reaches 4.89 and the percentage of extra bristles is 60.31. Do the extra-bristle factors come from the plus line?

Why a greater regression here than when crossed to the wild unless the plus line carries more plus modifiers than the wild fly? That the plus line does carry more plus modifiers than the wild is indicated by the fact that when the plus line is crossed to the wild some of the F₁ flies have extra bristles and that when these extra-bristled flies are inbred a strain of extra-bristled flies can be produced (table 22).

TABLE 23

A. *Plus line × line selected for extra bristles.*

Bristle number of parents	Bristle number of offspring											
	Female						Male					
	4	5	6	7	8	9	4	5	6	7	8	9
♀, 9 × ♂, 4	14	11	5		1		16	5	14	3	1	
♀, 9 × ♂, 4	18	9	3				5	11	14	13	4	
♀, 9 × ♂, 4	37	8	7	6		1	8	13	9	6	2	1
Totals	69	28	15	6	1	1	29	29	37	22	7	1

B. $F_1 \times F_1$.

Bristle number of parents	Bristle number of offspring																		
	Female								Male								Male, reduced		
	4	5	6	7	8	9	10	11	4	5	6	7	8	9	10	11	1	2	3
♀, 4 × ♂, 4	22	7	5	4	1				11	3	1	1	2					11	2
♀, 6 × ♂, 6	15	7	6	2	12	6	1	1	1	3	4	3	4		1		3	17	7
♀, 4 × ♂, 4	21	13	6	5	9	5	2		2	4	2	7	6	1	1	1	4	21	12
Totals	58	27	17	9	22	11	3	1	14	10	7	11	12	1	2	1	7	49	21

Table 23 gives the results of crossing the plus line to the line selected for extra bristles. Also the results of inbreeding the F₁ flies. Note the appearance of extra-bristled flies in F₁ and F₂. Note particularly that more of the F₁ males than females have extra bristles.

The cross of the plus and minus lines to the line selected for extra bristles gives interesting results. It should be recalled that the mutant "reduced" appeared in the sixth generation of the extra-bristle line. When the plus line is crossed to the extra-bristle line from which the mutant arose, some of the F₁ males and females have extra bristles (table 23). This might have been expected since extra bristles is partially dominant to the wild (table 24). When the minus line is crossed to the extra-bristle line we might expect the same result, but instead very few of the F_1 females, 5 out of 229, have extra bristles while many of the males, 40 out of 102, have extra bristles. In each of the five females only one extra bristle was present. Of the 40 males, 29 have one extra

TABLE 24

High-selection-line female × wild male.

Bristle number of offspring										
Female					Male					
4	5	6	7	8	4	5	6	7	8	9
135	135	89	32	5	124	102	58	20	10	1

Table 24 gives the results of crossing the high-selection line of my previous paper (PAYNE 1918) to the wild. Extra bristles is partially dominant.

TABLE 25

Minus line × line selected for extra bristles.

Bristle number of parents	Bristle number of offspring								
	Female		Male				Reduced male		
	4	5	4	5	6	7	0	1	2
♀, 0 × ♂, 8	78	3					44	36	10
♀, 10 × ♂, 0	50		35	12	4				
♀, 0 × ♂, 9	42	1					25	7	
♀, 9 × ♂, 0	54	1	27	17	5	2			
Totals	224	5	62	29	9	2	79	43	10

B. $F_1 \times F_1$.

Bristle number of parents	Bristle number of offspring														
	Female							Male				Male, reduced			
	4	5	6	7	8	9	10	4	5	6	7	0	1	2	3
♀, 4 × ♂, 5	55	8	6	3	3	3	1	22	4		1	33	12	1	1
♀, 4 × ♂, 4	67	16	10	6	3		1	27	6		1	22	13	7	4
♀, 4 × ♂, 6	19	4	3	1	7			9	6	2	4	7	2		
Totals	141	28	19	10	13	3	2	58	16	2	6	62	27	8	5

Table 25 gives the results of crossing the minus line to the line selected for extra bristles and the inbreeding of the F_1 flies. Note that very few of the F_1 females have extra bristles (5 out of 229). Forty out of 102 males have extra bristles. The ratio is in favor of the females in F_2.

bristle, 9 have two, and 2 have three extra bristles (table 25). In the case of the plus-line cross, 51 out of 120 F_1 females have extra bristles while 96 out of 125 males have extra bristles. Here then also more F_1 males than females have extra bristles. Why this difference in the F_1 males and females, and especially why the difference in favor of the males when in the extra-bristle line the females have on the average about one more bristle than the males? Can these results be explained on the basis of the conclusion that there is present in the minus line a single factor which inhibits or keeps the number of bristles below four, while in the plus line this same inhibitor is present, and in addition two modifying factors, one in the X chromosome and one in the third chromosome, which increase the bristle number to as high as four and in exceptional cases to five? I think they can.

It should be recalled that in the extra-bristle line there are at least two factors for extra bristles. One is in the X chromosome and the other in the third chromosome. Let us take the minus-line cross first. Our conclusion here was that there is a single inhibiting factor which keeps the number of bristles below four and we might even say below two as in the later generations of selection no flies had two bristles and only a very few had one bristle. The rest had none. If we use an extra-bristle-line female in the cross, all eggs will be alike, assuming of course that all factors for extra bristles are homozygous. They will have a factor for extra bristles in the X chromosome and one in the third. There will be two kinds of sperm in the minus-line male, the female-producing with the X chromosome carrying an inhibitor, the male-producing with no factors, either inhibitors or plus modifiers. The egg then with its two extra-bristle factors, when it meets the male-producing sperm of the minus-line male with no modifiers, produces males with extra bristles. On the other hand the X chromosome of the female-producing sperm carries an inhibitor. When it meets the egg, the zygote will have one X chromosome carrying a factor for extra bristles, the other carrying a factor for bristle reduction. Judging from the results, the factor for bristle reduction must neutralize the effect of the factor for extra bristles. The presence of an extra-bristle factor in one of the third chromosomes probably accounts for the few extra bristles which do appear. When the F_1 flies are inbred, there are as many extra-bristled females as males in F_2 (table 25).

When the plus line is crossed to the extra-bristle line both F_1 males and females have extra bristles. Here also, as previously mentioned, more males than females have extra bristles. We have concluded that in the plus line there are three factors present, one an inhibitor the same as in the minus line, and two other factors which modify bristle number in a plus direction. Reciprocal crosses gave similar results in F_1. For analysis let us use the extra-bristle female and the plus-line male. The eggs of the female parent will be the same as in the cross to the minus line. There will be one factor for extra bristles in the X and one in the third chromosome. In the plus-line male there will be two kinds of sperm, the female-producing with the X chromosome carrying the inhibitor and a plus modifier, and the third chromosome carrying a plus modifier. The male-producing sperm will carry only the plus modifier in the third chromosome. When the female-producing sperm meets the egg the zygote will have two X chromosomes, one from the extra-bristle line carrying a factor for extra bristles and one from the plus line carrying an inhibitor and also a plus modifier. This plus modifier was not present in the F_1 female of the minus-line cross. Of the third chromosomes the one from the extra-bristle line will carry a factor for extra bristles and the one from the plus line will carry a plus modifier. This plus modifier was not present in the F_1 female of the minus-line cross. Here then are two plus modifying factors present which were not present in the F_1 female of the minus-line cross and hence we should expect more extra bristles and more flies to show extra bristles. When the same egg with its two extra-bristle factors is fertilized by the male-producing sperm with one plus modifier in the third chromosome we have a zygote with one plus modifier in the X chromosome from the extra-bristle line. Of the third chromosomes one will carry an extra-bristle factor from the extra-bristle line and a plus modifier from the plus line. This zygote then has three plus modifying factors and no inhibitor. Hence more of the males than females have extra bristles. This F_1 male has one more plus modifier than the F_1 male from the minus-line cross and hence should have more bristles.

That the two plus modifiers in the plus-selected line may cause the appearance of extra bristles is indicated by crossing the plus line to a wild strain. This cross has been made to two separate wild strains and the result is the same in each. Table 22 gives the result of one such cross in which the plus-line male was used as one parent and the wild female as the other. Only a few extra-bristled flies appeared in F_1 (9 out of 448, or 2 percent). In F_2 however, 13 percent of extra-bristled flies is obtained. Inbreeding was continued for six generations using flies with extra bristles as parents. The percentage of flies with extra bristles was gradually increased from 2 percent in F_1 to 60.31 percent in F_7. The mean bristle number was increased from 4.02 in F_1 to 4.89 in F_7.

The minus-line male was also crossed to the same wild strain, in part to determine whether our conclusion that a single inhibiting factor is present would explain the results. If there are no plus modifiers here, we should expect no more extra bristles in F_1 and in later generations of inbreeding than we find in the wild strain. In fact we might expect to find fewer extra bristles in F_1 and F_2 if we have an inhibiting factor and no plus modifiers in the minus line. It should be recalled that when the minus line was crossed to the extra-bristle line only a very few of the F_1 females had extra bristles while more of the F_1 males had extra bristles. In the F_1 female the inhibiting factor in the X chromosome of the minus line apparently neutralized the effect of the extra-bristle modifier in the X chromosome of the extra-bristle line. In the cross to the wild strain we should likewise expect more of the F_1 males than females to have extra bristles. Such is actually the result shown in table 26. Of 240 F_1 females none had extra bristles. Three out of 237 F_1 males had extra bristles. This ratio, 3 to 474 is less than in the wild strain to which the minus line was crossed. Counts in the wild strain about the time the cross was made gave 327 flies with four bristles and 19 with five bristles. This is a percentage of 5.81 with one extra bristle. I may state that this wild strain had been bred in the laboratory about four years. When it was first brought into the laboratory a count of 613 flies gave 612 with four bristles and only one with one extra bristle. The continued inbreeding in the laboratory probably accounts for the presence of so many flies with extra bristles. In F_2 the percentage with extra bristles was increased to 2.35; in F_3 it dropped to 1.67; and in F_4 it rose to 6.30. Contrast this with 32.45, the percentage of extra-bristled flies in F_4 of the plus line crossed to the wild. Even the percentage of 6.30 is only slightly in excess of that found in the wild strain. I think we may safely conclude that the minus line has brought in no plus, modifiers.

Again it should be recalled that the mutant "reduced" from which both the plus- and minus-selected lines have come, appeared in the sixth generation of the line selected for extra bristles. In this sixth generation of the extra-bristle line 52.06 percent of the flies had extra bristles. The mean bristle number of the parents was 6.482; that of the offspring 4.88. The highest number of bristles on any one fly was nine. The extra-bristle line at this time must have carried factors for extra bristles. Since the reduced male appeared as a mutant from this line, it seems to me it would be possible and even probable that the mutant would carry these plus or extra-bristle modifiers. In the plus-selected line we have demonstrated the presence of two plus modifiers which push the number of bristles up to four and sometimes to five. Can these plus modifiers be the same as the plus modifiers in the extra-bristle line? I am inclined

TABLE 26

Wild ♀ × minus line ♂.

Generation	Bristle number of offspring											
	Female			Male			Male reduced			Totals	Number of flies with extra bristles	Percentage of flies with extra bristles
	4	5	6	4	5	6	0	1	2			
F_1	240			234	3					477	3	0.62
$F_1 \times F_1$	586	13	2	283	5	1	281	26	2	890	21	2.35
$F_2 \times F_2$	599	11		518	8					1136	19	1.67
$F_3 \times F_3$	130	18	1	97	3					349	22	6.30

Table 26 gives the results of crossing the minus-line male to a wild female and then inbreeding for three generations the flies with extra bristles. The percentage of extra-bristled flies was increased to 6.30 in F_4. This is about the same percentage found in the wild strain to which the minus line was crossed.

TABLE 27

Selection in the plus line after seven months in mass culture.

Generation	Bristle number of offspring										Mean	Percent with four bristles
	Female					Male						
	0	1	2	3	4	0	1	2	3	4		
1			2	39	119	1		28	43	66	3.51	62.08
2			5	53	237		7	48	104	152	3.53	64.19
3			7	29	176	1	4	39	67	86	3.50	54.05

Table 27 gives the results of three generations of renewed selection in the plus line after seven months of mass culture. The mean and percentage of four-bristled flies are brought back to what they were at the peak of the curve in the fifty-fifth generation.

to think that such may be the case, especially since the cross of the plus line to the wild gives extra-bristled flies in F_1 and an increasing number of extra-bristled flies in later generations of inbreeding (table 22). The extra-bristle factors must have been brought in by the plus line.

DISCUSSION

The present paper adds another link in the chain of accumulating evidence in favor of the multiple-factor hypothesis as an explanation of the effects of selection in bisexual forms. The conclusions reached agree with those of my former paper (PAYNE 1918) on selection for extra-bristle number on the scutellum. They also agree with the earlier conclusions of MACDOWELL (1915, 1917) on bristle inheritance in Drosophila; and finally with those of STURTEVANT (1918) on selection for high and low bristle number in the mutant race, dichaete. A review of the literature and a full discussion of all work bearing on selection need not be repeated here as this has been done by several writers recently. For a thorough and impartial discussion of the disputed points I refer the reader to the paper of STURTEVANT (1918).

A recent short article by CASTLE (1919) should be mentioned. His work on selection with the hooded rats has long stood as a stumbling block in the way of the multiple-factor hypothesis. With some new facts at hand he now interprets those results in favor of this hypothesis.

This change of CASTLE leaves JENNINGS and his students as the principal opponents of the multiple-factor hypothesis. In the unicellular forms they have been accumulating evidence of changes by minute variations and have interpreted their results in favor of the Darwinian theory of natural selection. In addition to these small variations they find occasionally larger ones which they call mutations.

Are these variations which JENNINGS and his students describe and which they use in selection, the same as the fluctuating variations of DARWIN? This question is somewhat difficult to answer as DARWIN did not specify exactly what he meant by such variations. It is the general opinion, I believe, that he referred for the most part to somatic variations. The small variations of JENNINGS and his students are certainly not somatic variations. In fact they speak of them as heritable variations. In other words they are germinal in origin. DARWIN also realized that variations, to be effective in the process of evolution, must be inherited, but he erred in thinking that practically all variations are inherited. Is then the selection of germinal variations, selection in the Darwinian sense? Or does this merely mean that among the large number of variations observed within a species some will be germinal and if such variations happen to be of marked advantage to the species they will be preserved? To be sure JENNINGS and his students have produced changes within their lines by selecting small heritable variations. Is this all the selection theory asks? Apparently to JENNINGS it is. Three question arise, however. First, are small heritable variations more numerous than larger heritable variations? Second, are these variations more apt to occur in the direction of selection than in other directions? Third, who or what determines whether a variation is large or small? It is impossible to answer the first question except in forms where actual breeding tests have been made, as a somatic variation is not distinguishable from a heritable one in any other way. In the protozoa, according to those who work with them, nearly all variations are small. This may be due in part to the fact that only the more extreme variations are called large. In other words it may be to a certain extent a matter of personal opinion whether a variation is said to be small or large. In the flies most of the heritable variations are large, at least large enough to be called mutations by those who work with them. To the second question it may be said that a mutation or a heritable variation may occur in the direction of selection or the reverse, and so far as I know is as apt to occur in one direction as in another. I think it may be said that we are more likely to see a variation in the direction of selection than in other directions because they are the ones we are looking for. This would be true particularly where the variations are small and where the eye is the judge of the differences. Also they are the ones which would be subjected to the breeding test. ZELENY (1920 b) has shown that by using the mutations which appear in the bar-eyed fly, facet number can be increased to full eye in one direction and to ultra bar in the other. As an example of a reverse mutation the reduced strain used for selection in the present paper arose as a mutant from the line selected for extra bristles. Third, as to whether a variation be large or small depends, as previously stated, to some extent upon the one who describes it. As an illustration JENNINGS (1917) describes the series of eye colors in Drosophila which behave as allelomorphs to white, as small heritable variations. He also describes the several modifiers of eosin eye color as small heritable variations. Those who have described this multiple-allelomorph system and the modifiers of eosin speak of them as mutations. Whatever you call them they did not occur in a graded series. What about the forms intermediate between the large and small variations? Would they be small heritable variations or would they be mutations? What I wish to emphasize is that it depends to a large extent upon the individual who describes the variation whether it be small or large, and that there is no sharp line of demarkation between the two; they intergrade. Why then make a distinction between them? To me there is no difference so far as evolution is concerned. They are one and the same thing. Both are genotypic changes and as such are inherited.

Are then the views of JENNINGS and his students and the multiple-factor hypothesis mutually exclusive? It seems to me they are not. In the multiple-factor explanation of selection it is not necessary to assume that all modifying factors are present at the beginning of selection. Some of them may arise as mutations during the course of selection. This was indicated in my earlier experiments (PAYNE 1918). It has been demonstrated by ZELENY (1919) to occur in selecting for facet number in the bar-eyed mutant. ZELENY has also shown that the principal gene itself may change as in the reversal of bar to full eye and in the change from bar to ultra bar. Are not such mutations germinal variations, and how do they differ from the germinal variations of JENNINGS? The only difference I can see is in the size of the variation. When we can reduce the differences between two opposing theories to a question of the size of the variations, it seems to me the differences disappear entirely. Why not call all genotypic variations, mutations, or if we object to such an interpretation for the word, mutation, leave it out of our discussion and use the expression, "genotypic variation"? Certainly no one will object to saying that a genotypic variation may be large or small.

JENNINGS does not specify whether the heritable variation is due to a change in a single gene or whether through the origin of modifying factors. He means to imply that both methods may function, I think. In fact JENNINGS (1917) seems to think that it matters but little how the variations arise. The essential fact is that they do arise and this is all the selection theory asks.

Adherents of the multiple-factor hypothesis have usually assumed that selection is effective for a few generations and then ceases. This is not necessarily the case, as in the strain selected for extra bristles (PAYNE 1918) selection was effective for 29 generations. In the present paper the peak of the curve in the plus line was not reached until the fifty-fifth generation. So if genotypic changes occur within the line in the direction of selection either as changes within the principal gene or as factors which modify the character in question there is no reason why selection should not continue to be effective for long periods of time.

CONCLUSIONS

1. The mutant "reduced" appeared in the sixth generation of the line selected for extra bristles. The name applies to the number of bristles on the scutellum. Four is the wild-type number. In this mutant the number may be four, three, two, one, or none at all.

2. The strain has been separated very clearly into minus and plus lines by mating flies with no bristles on the one hand and flies with four bristles on the other.

3. In the minus line selection was effective for 17 generations. During this time the mean was reduced from 0.504 to 0.004 and the percentage of flies with no bristles was gradually increased from 64.61 to 99.52. From the seventeenth to the sixty-fourth generation the mean and the percentage of zero flies fluctuated slightly, but certainly there was no further genetic change after the seventeenth generation. While in the fortieth generation all the flies were bristleless, it was impossible to produce a strain without bristles.

4. In the plus line the mean was increased from 1.95 in the first generation to 3.25 in the eighteenth. The percentage of four-bristled flies was increased from 7.20 in the first generation to 46.90 in the eighteenth. From the eighteenth to the forty-sixth generation the mean and percentage of four-bristled flies fluctuated considerably. With the forty-sixth generation both began to rise and continued until the mean reached 3.52 and the percentage of four-bristled flies reached 64.25 in the fifty-fifth generation. From the fifty-fifth to the sixtieth there was a decline. The mean went to 3.11 and the percentage of four-bristled flies to 40.56.

5. The decline in the mean and the percentage of four-bristled flies in the last six generations of selection was probably due to weakness in the line. This conclusion is strengthened by the fact that after the strain was continued in mass culture for eight months renewed selection brought back immediately the mean and percentage of four-bristled flies to approximately what they were in the fifty-fifth generation.

6. Back selection in both lines was ineffective.

7. In mass cultures for eight months the two lines have remained what they were at the end of selection. They are not only somatically different, but they are genetically different.

8. By linkage and crossing over it has been demonstrated that in the minus line a single sex-linked factor is present. This factor seems to act as an inhibitor.

9. By the same method it has been demonstrated that in the plus line there are present at least three factors and probably more. One is the inhibitor present in the minus line. In addition there is a plus modifier in the X chromosome near miniature and a plus modifier in the third chromosome near sepia.

10. When the plus-line female is crossed to the minus-line male, there is a greater reduction in the bristle number of the F_1 males than when the plus-line female is crossed to the wild male. This can be explained on the assumption that the wild male has plus factors not present in the minus-line male. It also leads me to believe that the four-bristled condition in the wild fly is not due to a single factor.

11. When the minus-line female is crossed to the plus-line male, fewer F_1 males have no bristles than when the minus-line female is crossed to the wild. This indicates that the plus line has more plus modifiers than the wild fly. The number of bristles then in the plus line must be kept below those of wild flies by the presence of the primary inhibiting factor, reduced.

12. When the plus line is crossed to the wild some of the F_1 flies have extra bristles. By continued inbreeding of these extra-bristled flies a strain of extra-bristled flies can be produced. These extra-bristle factors must have come from the plus line. This seems all the more probable when it is recalled that the reduced strain arose from the sixth generation of the line selected for extra bristles. This also leads me to suspect that the plus modifiers of the plus line may be the extra-bristle modifiers of the extra-bristle line.

13. Selection for extra bristles in a second strain has increased the mean to 5.17 in the fifth generation. In the sixth generation it declined to 5.09. No flies have more than seven bristles and only a few have this number.

14. Low temperature increases bristle number.

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GENETIC STUDIES ON THE PROTEIN CONTENT OF MAIZE

E. M. EAST

Harvard University, Bussey Institution, Forest Hills, Massachusetts

AND

D. F. JONES

Connecticut Agricultural Experiment Station, New Haven, Connecticut

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INTRODUCTION

Agricultural crops owe their popularity to a variety of qualities. If they will keep for long periods, if they can be stored economically and shipped easily, if they suit all palates, their usefulness is naturally enhanced. But, other things being equal, their content of particular desirable constituents determines not only their true worth, but their commercial value. Such a statement is no more than a platitude when it concerns plants grown for fibers, drugs, dyes, tannins, rubbers, or essential oils. In the great food plants, the matter is not so obvious; yet data collected some months ago by one of us, indicate a rather striking class difference and noteworthy uniformity within each class in the prices people have come to pay for carbohydrates, fats, proteids and vitamine carriers, when correction is made for the variables noted above.

Naturally, appreciation of differential values in plant constituents has led to many plant-breeding projects in which the chief aim has been to develop the desirable quality rather than to increase the yield of the crop. One cannot criticize efforts to raise the quantity and quality of fiber in cotton and flax, or the yield of rare oils and drugs. In other cases one cannot be so certain that such efforts are advisable. It may be true, for example, as maintained by HOPKINS and SMITH, that there is demand for maize varieties rich in high or low protein or in high or low oil. It is possible, however, that the best economic practice is to grow other plants for these particular purposes. If the difficulties in the way of breeding wheat and maize for high protein content or maize for high oil content are extreme, it is probably wiser to obtain proteid concentrates or commercial oils from plants naturally rich in these substances. Nevertheless facts regarding the inheritance of such chemical constituents as protein are always valuable, and the writers submit this paper hoping that the results set forth have some present value to genetic theory and the possibility of future value to agricultural practice.

The work is based upon an experience of nearly twenty years. From 1900 to 1905, familiarity with the problems involved was gained by contact with the maize-breeding projects of the ILLINOIS AGRICULTURAL EXPERIMENT STATION. In 1906 mass-selection experiments were started at the CONNECTICUT AGRICULTURAL EXPERIMENT STATION. It was soon apparent, however, that information additional to that secured by the ILLINOIS AGRICULTURAL EXPERIMENT STATION was not likely to be obtained until more was known about the inheritance of simpler characters. Accordingly the investigations on protein inheritance were held in abeyance for several years. Since 1909, however, the work has been prosecuted with some vigor by Professor H. K. HAYES now of the UNIVERSITY OF MINNESOTA and by the authors, as a coöperative project between the CONNECTICUT AGRICULTURAL EXPERIMENT STATION and HARVARD UNIVERSITY. The funds for the plat work were furnished wholly by the first-named institution, the pedigree cultures were grown upon its experimental farm, and the chemical determinations were made within its laboratories,—in earlier years by the authors, in later years by the staff of the Chemical laboratory. Current analyses of the results and plans for each year from 1909 to 1917, were made first by H. K. HAYES and E. M. EAST and later by D. F. JONES and E. M. EAST during the winters in the laboratories of the BUSSEY INSTITUTION of HARVARD UNIVERSITY.

RESULTS OF PREVIOUS WORK

It would serve no useful purpose to describe in detail the early plant-breeding work wherein the chief object was to enhance the value of a plant by increasing the production of a particular chemical constituent. The classical example, and one of the earliest projects for breeding plants on a large scale, is the work of the French chemists and agriculturists on the sugar beet, inaugurated in the early part of the nineteenth century by the great Corsican, and carried on continuously since that time both in France and in Germany. Similar work on the sugar cane has been carried on in Java and to a limited extent in Cuba. No other comparable work of such magnitude or of such commercial importance exists, yet each year sees the initiation of some plan of this kind on a small scale. There are schemes for increasing and decreasing protein in wheat and maize, for increasing oil in maize, peanuts, castor beans and soy beans, for obtaining greater yields of the essential oils used in perfumes and of the valuable ingredients in certain drug plants. And recently both English and American companies have undertaken work designed to augment the yield of rubber latex in *Hevea brasiliensis*.

Few of these projects have resulted in any supposed or actual increase in genetic knowledge. In nearly every case mass selection has been practised with no more powerful tool of knowledge than the empiric formula "like tends to produce like." Even the numerous German contributions to literature on the sugar beet have had no effect on current genetic thought. They have resulted in a better knowledge of the varying composition of different parts of the root and in the effects of factors of environment on the elaboration of sugar, but even today one cannot say with certainty whether new variations have occurred which aid in the production of higher sugar content. Elimination of lines with a low sugar content, and accumulation of favorable genetic factors by segregation and recombination may account for everything in the sugar beet, though one hears much about the *change* in the ability of the plant to produce sugar. It may be doubted whether the extreme individual beet is higher in sugar today than in the year 1800, but there are more of these high individuals.

An exception to these remarks is the work carried on since 1896 at the ILLINOIS AGRICULTURAL EXPERIMENT STATION, where the primary object has been to establish commercial strains of maize characterized by high or low protein content, or by high or low oil content, but where the idea of contributing to genetic knowledge has never been allowed to lapse. It is natural, therefore, that these experiments should be described in some detail in this paper.

In 1892, JENKINS and WINTON compiled the published analyses of American feeding stuffs. Among them were analyses of maize seeds produced by plants of different varieties grown in various parts of the country. The range and average of protein content calculated to water-free material are shown in table 1. These reports of protein are in reality determinations of total nitrogen multiplied by the factor 6.25. That this factor is sufficiently correct for all practical purposes is demonstrated by the work of CHITTENDEN and OSBORNE (1892) where the weighted average percent of nitrogen in the different proteid bodies was found to be 16.00.

TABLE 1

Percentage protein content of maize seeds in water-free material.

Type	Number of samples	Maximum	Minimum	Average
Dent	86	13.8	8.2	11.5
Flint	68	14.0	7.7	11.8
Sweet	27	17.0	10.3	12.8
Pop	6	14.4	11.0	12.5
Soft starch	5	15.5	9.5	12.5

There are various proteids in maize as these investigations show:

	Percent
1. Proteose soluble in water	0.06
2. Very soluble globulin	0.04
3. Maysin soluble in dilute salt solution	0.25
4. Edestin soluble in concentrated salt solution	0.10
5. Zein soluble in alcohol	5.00
6. Proteids soluble in dilute alkalies	3.15
7. Proteids insoluble in these solvents	1.05

Thus zein is the important proteid, comprising over 50 percent of the total. Unfortunately zein lacks the essential amino acids, glycocoll, lysine and tryptophane, and contains relatively small amounts of arginine and histidine; hence it cannot be used as the sole proteid food in building up animal tissues, as has been shown by Osborne and his co-workers in numerous investigations.

Variations comparable to those of table 1 were found in all the constituents of maize, and gave Hopkins the basis for starting the Illinois Agricultural Station experiment. Admittedly, a considerable portion of these deviations were due to varying environmental conditions, but he was satisfied that there were heritable differences in composition which could be made the basis of selection. He was not able to prove this at the time, but offered some presumptive evidence in the shape of individual analyses of 50 selected ears of Burr's White maize grown on a field having particularly uniform soil conditions. The frequency distribution of proteins and of oil contents (table 2), showing, as they did, deviations as great as the compiled analyses of maize grown under extremely varied conditions, were accepted as indicating differences due to heredity.

Table 2

Frequency distribution of percents of protein and of oil in 50 ears of Burr's White maize grown under uniform soil conditions.

Classes	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0
Distribution of protein	2	3	2	6	8	8	6	9	2	3	0	1

Classes			4.0	4.3	4.6	4.9	5.2	5.5	5.8	6.1		
Distribution of oil			5	9	11	16	5	3	0	1		

Hopkins assumed, with little biological, but with considerable pragmatical justification, that the ear of the maize plant may be taken as a unit. Marked variations were found when samples of seed were taken from top, middle or butt seeds, but several samples of three rows of seeds taken from the whole length of the ear showed very small deviations (table 3). Similarly the comparative uniformity of the protein content of single seeds from the same ear—after 3 years of selection for protein—was later taken as warranting the conclusion that the composition of the ear is approximately uniform throughout (table 4).

Starting with these assumptions, 163 ears of Burr's White maize were analyzed (Hopkins 1899). Considering only the protein and the oil contents, the distribution of the analyses was as follows (table 5). From selected ears of this lot, breeding in four directions was begun,—(1) high protein, (2) low protein, (3) high oil, (4) low oil. In general the method was to select twenty-four ears of each type by chemical analysis every year, and to continue each line in an isolated breeding-plot by the ear-row method. The main criterion of selection was the chemical composition, but naturally some attention was paid to the appearance of the ear and to its yield as measured by the resulting progeny.

TABLE 3

Deviation in percentage of protein in random samples from single ears.

Ear number	Sample from		
	Tip	Middle	Butt
1	11.77	12.24	12.39
2	11.98	12.49	13.06
3	9.70	10.08	10.48
4	10.60	11.04	11.00
5	10.82	11.33	11.30
	1st 3 rows	2nd 3 rows	3rd 3 rows
1	10.77	10.96	10.69
2	11.99	12.03	12.14
3	10.10	10.16	10.18
4	10.46	10.26	10.08
5	11.20	10.64	10.86

TABLE 4

The percentage protein content of individual seeds on the same ear.

Seed number	Ear number				
	1	2	3	4	5
1	12.46	12.17	11.53	7.45	7.72
2	12.54	12.94	12.32	7.54	8.41
3	12.44	12.51	12.19	7.69	8.37
4	12.50	13.42	12.54	7.47	8.31
5	12.30	13.12	12.14	7.74	8.02
6	12.49	14.49	12.95	8.70	8.76
7	12.50	13.21	12.84	8.46	8.89
8	12.14	13.43	12.04	8.69	9.02
9	12.14	13.16	12.75	8.86	8.96
10	12.71	14.05	—	8.10	8.89

The desire to keep up the yield and to preserve a good physical type, as well as the fact that selection was made only through the mother, obviously prevented a rapid shift of type; yet the results were rather remarkable. Through the kindness of Dr. L. H. SMITH we are enabled to quote the gross averages up to the year 1919.

In table 6, the crop averages for the high-protein and the low-protein plots are presented. There is an increasing difference in the protein content of the two strains which continues until the high-protein type contains 8.17 percent more protein than the low-protein type. When the data are shown graphically (figure 1), two facts stand out impressively. The shift in the average is rapid at first; but becomes slower and slower, until the shape of the fitted curves are changed from concave to convex at about the sixth or seventh generation. Nevertheless there is a continuous shift of the average, and apparently the end is not in sight. Second, the influence of environment is very marked. The protein content of the two plots goes up or down according to the season, but always the difference between the two increases.

TABLE 5

Distribution of protein and oil in the 163 ears of maize with which the Illinois Agricultural Experiment Station maize-breeding investigations were started.

Oil, percent	Protein, percent													Total number of ears
	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	
3.8								1						1
4.0			2	2			2	3	1	1	1			12
4.2				3	2	3	3	2	3		2			18
4.4				3	4	2	7	3	2					21
4.6			2	1	1	8	5	8	4	3				32
4.8	1	1		5	6	3	7	2	4	5				34
5.0		1		2	1	3	3	5	4		1			20
5.2				2	3	2	2	1		3	1			14
5.4						2								2
5.6				1	1	1	1		1		1			6
5.8							1						1	2
6.0					1									1
Total number of ears	1	2	4	19	19	24	31	25	19	12	6	0	1	163

Table 7 shows the figures for the high-oil and the low-oil plots in the same way. The high-oil strain in 1918 has finally reached the remarkable figure of 9.35 percent, while the low-oil type contains only 1.87 percent. Graphically the curves (figure 2) for the changes in oil contents show rather more regularity than is the case with the protein. The rate of change is comparatively constant, seasonal differences apparently having little to do with the matter.

The remarkable results obtained in these experiments have been the object of much comment; and, as BABCOCK and CLAUSEN (1918) point out, the theoretical interpretations of the rôle which selection played, have been varied. The conclusions of Hopkins and Smith (1903–1917), of Davenport (1908) and of Castle (1916), have been similar in that they seem to attribute a peculiar creative power to selection which meets with a certain response on the part of the plant. The reason for these conclusions appears to be in part an adherence to the Darwinian idea that all fluctuations are heritable and that continuous selection is therefore always effective in shifting the type; in part a scanty appreciation of the results of other pedigree-culture work; and in part a failure to realize that the unit of selection is the seed and not the ear, combined with a lack of appreciation of the variables which come into play when a system

Table 6

Results of selecting maize for high and for low protein content at the Illinois Agricultural Experiment Station. Average percent protein in crop each generation.

Year	High strain	Average for period	Low strain	Average for period	Difference	Difference for period
1896	10.92		10.92			
1897	11.10		10.55		0.55	
1898	11.05		10.55		0.50	
1899	11.46		9.86		1.60	
1900	12.32	11.37	9.34	10.24	2.98	1.13
1901	14.12		10.04		4.08	
1902	12.34		8.22		4.12	
1903	13.04		8.62		4.42	
1904	15.03		9.27		5.76	
1905	14.72	13.85	8.57	8.94	6.15	4.91
1906	14.26		8.64		5.62	
1907	13.89		7.32		6.57	
1908	13.94		8.96		4.98	
1909	13.41		7.65		5.76	
1910	14.87	14.07	8.25	8.16	6.62	5.91
1911	13.78		7.89		5.89	
1912	14.48		8.15		6.23	
1913	14.83		7.71		7.12	
1914	15.04		7.68		7.36	
1915	14.53	14.53	7.26	7.74	7.27	6.79
1916	15.66		8.68		6.98	
1917	14.44		7.08		7.36	
1918	15.48		7.31		8.17	

of breeding by selection through the mother is practised. DAVENPORT and RIETZ (1907), for example, studying the four strains by statistical methods after ten years of selection, use the ears as units of discussion and conclude that "the variability was not sensibly reduced during the

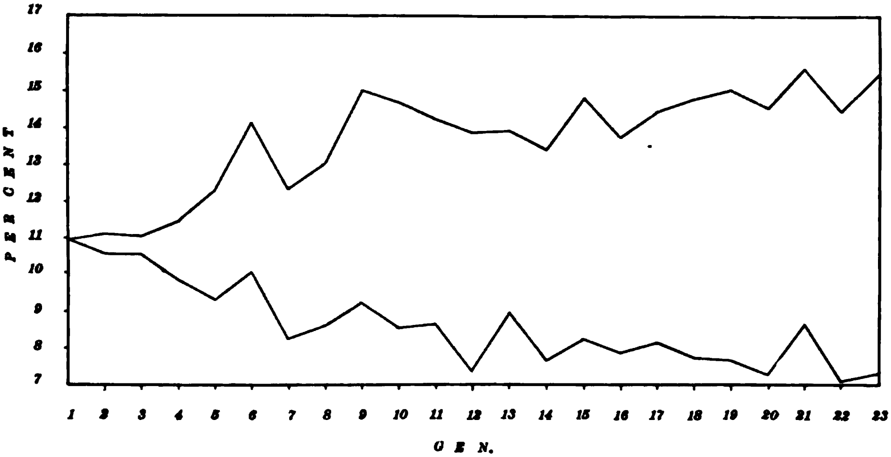

FIGURE 1.—Graphical representation of the results of the ILLINOIS AGRICULTURAL EXPERIMENT STATION in selecting maize for high protein and for low protein.

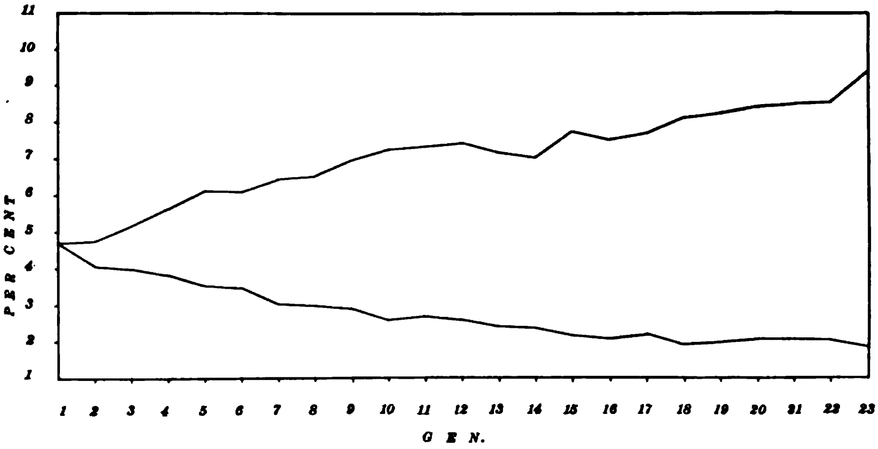

FIGURE 2.—Graphical representation of the results of the ILLINOIS AGRICULTURAL EXPERIMENT STATION in selecting maize for high oil and for low oil.

ten years of rigid selection." Their study of the data, led them to believe that "after great improvement has been secured there is still left abundant variability on which to base future selection, and that if the limits of improvement are ever reached it will be for some reason other than the failure of variability." In other words, "the effect of selection is to shift the type without greatly altering variability."

These data do indeed show that the effect of selection has been to bring about the production of four types as distinct in their physical characteristics as in their chemical composition; but if one realizes the slowness of obtaining particular Mendelian recombinations when indiscriminate pollination is resorted to, and appreciates the statistical result when the ears are regarded as the units of heredity, it is clear that other interpretations are possible. In passing it may be said that the ear is a population of seeds; and it can be shown graphically (figure 3) that selection may take place for many generations, reducing the variability

TABLE 7

Results of selecting maize for high and for low oil content at the Illinois Agricultural Experiment Station. Average percent oil in crop each generation.

Year	High strain	Average for period	Low strain	Average for period	Difference	Difference for period
1896	4.70		4.70			
1897	4.73		4.06		0.67	
1898	5.15		3.99		1.16	
1899	5.64		3.82		1.82	
1900	6.12	5.41	3.57	3.86	2.55	1.24
1901	6.09		3.43		2.66	
1902	6.41		3.02		3.39	
1903	6.50		2.97		3.53	
1904	6.97		2.89		4.08	
1905	7.29	6.65	2.58	2.98	4.71	3.67
1906	7.37		2.66		4.71	
1907	7.43		2.59		4.84	
1908	7.19		2.39		4.80	
1909	7.05		2.35		4.70	
1910	7.72	7.35	2.11	2.42	5.61	4.93
1911	7.51		2.05		5.46	
1912	7.70		2.17		5.53	
1913	8.15		1.90		6.25	
1914	8.29		1.98		6.31	
1915	8.46	8.02	2.07	2.03	6.39	5.99
1916	8.50		2.08		6.42	
1917	8.53		2.09		6.44	
1918	9.35		1.87		7.48	

of each line-bred family in a marked degree, without necessarily reducing the variability of averages of population samples which are obtained by analyzing ears. It is shown in figure 3, for instance, that a normal frequency surface may be constructed in which the variability in one direction—measuring a series of samples—may remain the same, though the variability of the sub-population making up each sample, is very different.

The interpretation noted above has been given up by CASTLE (1919) because of the steadily increasing evidence that recombinations of Mendelian factors account for the results obtained, in a simpler and more helpful manner.

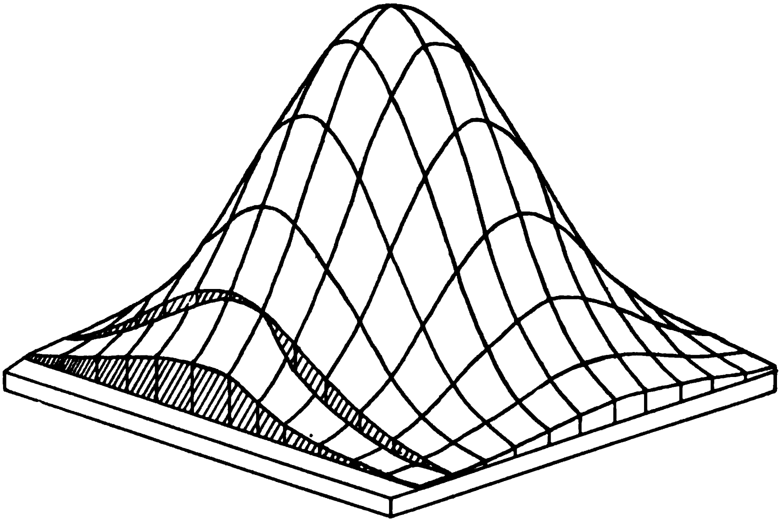

FIGURE 3.—A normal frequency surface showing how the dispersion coefficient may be changed in one direction without affecting it in the other direction.

A Mendelian interpretation of the Illinois results, isolation of various combinations of hereditary factors, was first suggested by EAST (1910), on the general basis that it was more plausible to have an analysis of these facts in keeping with modern genetic interpretations of analogous phenomena, and for the specific reason that the fitted curves showed a retardation in the effects of selection.

This stand was strongly supported by an analysis of the pedigrees of the four Illinois strains after ten years selection, made by SURFACE in 1911. He found that the 24 "High-protein" ears selected for planting in the eleventh generation, all traced back to one original ear, No. 121; of the "Low protein" ears, 20 traced back to No. 106 and 4 to No. 107;

of the "High oil" strain, 12 ears came from No. 111, 4 ears from No. 114 and 8 ears from No. 118; and of the "Low oil" strain 16 ears originated from No. 106 and 8 ears from No. 110. Thus in the eleventh generation all of the 96 ears of the four strains traced back to 8 ears of the original Burr's White, a rather convincing demonstration that the results of selection were mainly the accumulation of hereditary complexes effective in various ways, though of course no one could maintain that mutations had not ensued during this lapse of time.

Other experiments modeled along lines similar to these have been carried on at various agricultural experiment stations since the first report of the Illinois investigations, but so far as we know only one resulted either in new facts or in a new point of attack. We refer to that of PEARL and BARTLETT (1911). In this study a cross was made between a white sugar corn and a yellow dent, and F_2 seeds of the four classes, yellow dent, white dent, yellow sugar and white sugar, were analyzed and the results compared with those obtained from seeds of the parental and F_1 generations. Moisture, nitrogen, ether extract, ash, crude fiber, pentosans, sucrose, dextrose and starch were determined. No discussion of the relative accuracy of these determinations was made, but it was thought that high moisture and high starch dominated the alternative conditions, low moisture and low starch, while in the remaining constituents the lower percentage dominated the higher percentage. Segregation was obvious in every case. It was not shown, and probably the authors would not now maintain, that single factors determined the difference between "high" and "low" content of any of these complexes; moreover, matters other than simple segregation in the usual sense must be taken into consideration in such a genetic analysis, as we shall show; but the authors deserve great credit for bringing out the fact that the seed and not the ear must be taken as the unit in any such study.

THE INHERITANCE OF PROTEIN IN MAIZE

The problem

The genetic problems involved in an effort to change the chemical composition of maize by breeding cannot be understood clearly unless the elementary botanical facts connected with seed formation are borne in mind. This would seem to be, and ought to be, an unnecessary observation; yet a careful survey of the statements made by previous investigators leads one to believe that ignorance or carelessness regarding these facts has led to numerous erroneous conclusions.

One should expect the composition of the maize seed to be influenced both by the genetic constitution of the mother plant and by the environment under which it develops, but it should not be forgotten that the grain itself, speaking botanically, is in a sense a young zygote having characters of its own derived from the gametes from which it is formed. Classified according to their origin, however, there are these distinct parts to the seed,—the pericarp, a maternal tissue, the embryo formed by the union of the egg with the first male nucleus, and the endosperm formed by the union of the second male nucleus with the so-called endosperm nucleus—a fusion product of two embryo-sac nuclei. The line of hereditary transmission is confined to the gametes produced by the plant maturing from the embryo, but the composition of the seed is determined largely by the composition of the endosperm which forms about 80 percent of each individual kernel. Now the cytological and the pedigree-culture evidence are in agreement that the above method of seed formation is so rigid in the species under consideration that no one has been able to establish an exception. These experimental methods have also demonstrated (see EAST 1913), first, that from the chromosome standpoint the embryo is a $2x$ body and the endosperm a $3x$ body; second, that the two "male" nuclei on the one hand and the three "female" nuclei on the other hand, have respectively the same genetic composition. If a male nucleus entering into endosperm formation bears a factor through which a particular character develops, therefore, one may rest assured that the "brother" nucleus entering into the formation of the embryo, will bear the same factor. And the same is true of the three "female" nuclei. Nevertheless some complications arise, due to this double-fertilization process, which make the various genetic phenomena involved somewhat difficult to analyse, although the basis upon which such analysis must depend is quite clear.

For example, in the earlier investigations on inheritance of maize-endosperm characters, such characters as the yellow ether-soluble pigment, the blue and red anthocyans of the aleurone cells, and the presence and absence of starch development, it was found that the endosperm could be considered to be identical with the embryo without error. The dominant characters seemed to show the same degree of dominance, a degree approaching perfection, no matter whether they entered from the male or the female side. In other words, a single nucleus contributing certain factors from the male side, seemed to exert the same influence on development as a double ($2x$) nucleus entering from the female side. HAYES and EAST (1915), however, found that this simple behavior was not characteristic of every character. In the starch differences causing the chief distinction between the floury types and the horny or translucent types, dominance followed the maternal side. The two nuclei coming in from the embryo sac seemed to have a cumulative effect. Corroboration of this phenomenon was recently made by JONES (1919) on another type of starch difference.

The fact that protein is contained in each of these three types of tissue is a further fact that complicates the genetic problems. HOPKINS, SMITH and EAST (1903) found that after four or five years' selection for high protein and for low protein, the high-protein and the low-protein strains had been differentiated physically to such a degree that the embryos and the amount of corneous starch in the high-protein strain were considerably greater than in the low-protein strain. In a single selected low-protein ear the pericarp and remains of the vestigial glumes comprised 6.67 percent, the endosperms 83.73 percent, and the embryos 9.59 percent of the total. Similarly, in a high-protein ear the maternal tissue comprised 7.71 percent, the endosperms 80.37 percent, and the embryos 11.93 percent. The nitrogen was very low in the maternal tissues and probably did not consist largely of proteid nitrogen. The actual percentage of protein in the embryos was high but did not differ very much in the two types, being 19.91 percent in the low-protein ear and 19.56 percent in the high-protein ear. The greatest difference came in the endosperm, where the aleurone layer (probably contaminated with starch) of the low-protein ear contained 19.21 percent protein as compared with 24.58 percent in the high-protein, and the corneous starch (37.15 percent of the seeds by weight) contained 8.12 percent in the low-protein as compared with 10.99 percent in the high-protein ear (44.89 percent of the seeds by weight).

It is not certain what relative changes in the kinds of protein contained were made by the isolation of these strains, but from OSBORNE and CLAPP'S (1908) analyses of ordinary maize and maize from the high-protein plot of the ILLINOIS AGRICULTURAL EXPERIMENT STATION, one would suppose that the proteins of the endosperm had been increased to a greater degree than the proteins of the embryo.

These various facts regarding the origin of the maize seed and the composition of its various parts have been kept in mind, and the problem of changing the protein content has been attacked in various ways. The results obtained will be discussed seriatim.

It is to be understood that all chemical determinations were made by the methods approved by the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

Variation in the protein content of individual seeds

The method of work at the ILLINOIS AGRICULTURAL EXPERIMENT STATION was founded on the fact that in certain ears tested, triplicate samples of three rows of seeds taken throughout the length of the ear gave very uniform analyses, though rather marked variations in analysis were noted when ears were sampled at the tip, middle and butt. Under the circumstances under which the Illinois analyses were made this was perhaps to be expected. It is not surprising, for example, to find variations in the chemical constitution of the seeds at the tip, the middle and the butt, for the seeds in these various regions differ in average size, are pollinated successively from butt to tip because of the maturation of the silks in this order, mature at slightly different times, and presumably may be expected to receive somewhat different amounts of nourishment from the parent plant owing to the spike-like mode of development of the ear. For the same reasons, it is to be expected that samples taken throughout the length of the ear will more truly represent the whole ear. Duplicate samples taken in this manner should be similar. At the same time it should be remembered that analyses of duplicate samples taken in this way, tell one nothing concerning the variation shown by individual seeds or the individual potentialities they carry.

For actual use, no better method of selection can be suggested, yet if individual seeds do show a notable variation due to varying zygotic composition, it will depend largely on the heterozygosity or homozygosity of the genes present, whether progress by the mass-selection method will be rapid or slow. In other words, of two ears of 15 percent protein selected for their high protein content, the one might have a coefficient of variation of 7 percent, the other of 16 percent. In mass selection the ear uniformly high in protein would undoubtedly give the best results, for it is likely that there would be 7-percent or 8-percent seeds, both in a phenotypic and a genotypic sense, in the ear with the high dispersion coefficient. The effect of pollen from the plants these seeds would produce can easily be imagined. On the other hand, if self-pollination were practised, the seeds from the more variable ear would hold out the greatest hopes for improvement, for it is probable they would run as high as 19 or 20 percent.

These suppositions can be illustrated from results actually obtained by analysis of individual seeds. In 1907 the ILLINOIS AGRICULTURAL EXPERIMENT STATION kindly sent some ears of their high-protein and low-protein strains to the CONNECTICUT AGRICULTURAL EXPERIMENT

STATION. These strains after the ten years' selection had already become markedly differentiated. The high-protein strain as compared with the low-protein strain had smaller seeds, larger embryos and a much greater percent of corneous starch. A cross was made between two plants, high-protein female by low-protein male, and a series of seeds of the following types were analyzed: (1) seeds from one ear each of selfed high-protein and of selfed low-protein, these being ears from sister plants of those used in the cross, (2) seeds from the ear produced by the immediate cross, i.e., F_1 seeds, and (3) seeds from each of two F_1 ears bearing F_2 seeds. These analyses were made for another purpose at the time, hence some data which might be useful at the present time are lacking; but they will serve our purpose. Protein contents are calculated to dry basis as has always been our practice, but it was impractical in this case to actually dry each sample by the laboratory method. The ears were air-dried in a steam-heated room, and a single moisture determination made for each ear. Moisture determinations made in this way were about 8.3 percent. Since the variation was small, the range being 1.2 percent, it may be assumed that the method was very accurate for our purpose. The samples were taken in spiral fashion around the ear, as fair a method as could be devised. The results are shown in table 8, where the frequency distribution of protein is tabulated in one-half percent classes.

Because of the small number of individuals and large experimental error involved, and because one cannot feel certain that analyses from a single cross represent accurately the conditions usually found in similar crosses, one should be careful not to draw any hard and fast conclusions from these data. The ranges of variation in the various distributions seem to be rather small, and thus corroborate HOPKINS's conclusions cited previously. The F_2 seeds, however, show a somewhat greater range. Turning to table 9 where the statistical constants are shown, one is somewhat surprised to find the rather high variability of the low-protein type. Judging from the appearance of the curves plotted from the figures given, it seems reasonable to suppose that with larger numbers a greater difference in variability between the parents and the F seeds would be found; but, of course, this is a mere surmise. One conclusion, at least, is permissible. It is clear to anyone who has had experience in studying dispersion coefficients of pedigree cultures, that the phenotypic differences between seeds on the same ear are not what would have been expected had the genotypic differences of these seeds been expressed in the composition of the individual grains. In other words, only a very

TABLE 8

Variations in protein content in individual seeds (dry basis).

Source of seeds	Class centers in percent																				
	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5
Ear A1, high protein....											1	1	7	13	18	14	11	4	1		
Ear B1, low protein.....	1	4	8	22	23	8	5	2	1												
Ear (A2 × B2), F_1 seeds										2	4	7	13	26	13	5	1	2			
Ear (A2 × B2)1, F_2 seeds									1	0	1	8	4	6	8	18	15	3	2	1	1
Ear (A2 × B2)2, F_2 seeds								1	2	2	5	7	11	17	16	12	7	3	1		

small portion of the potentialities which a seed may have inherited is expressed in the quality of the individual seed itself by the time it has finished its growth preparatory to the resting period.

The obvious general conclusion from the data thus far considered, therefore, is this: The seeds of maize vary in the transmissible hereditary factors which control the development of the various proteids in the different parts of the seed. These factors, transmitted in the usual manner, must be the basis of all change in composition through breeding. At the same time, there are extraordinary practical difficulties involved. The greater part of the protein is contained in the endosperm, where, though nuclei presumably duplicating those which form the embryo come together, there is the difference that the contribution from the maternal side is twice that of the paternal side. In addition the composition of the seed as a whole is dependent on extremely complex conditions.

TABLE 9

The statistical constants of the frequency distributions shown in table 8.

Source of seeds	Number	Mean	Standard deviation	Coefficient of variation
Ear A1, High-protein	70	13.63±0.06	0.78±0.04	5.72±0.33
Ear B1, Low-protein	74	8.32±0.06	0.72±0.04	8.65±0.48
Ear (A2 × B2), F_1 seeds	73	12.91±0.06	0.78±0.04	6.04±0.34
Ear (A2 × B2)1, F_2 seeds	68	13.71±0.09	1.12±0.06	8.17±0.47
Ear (A2 × B2)2, F_2 seeds	84	12.08±0.08	1.08±0.06	8.94±0.47

First, all external factors affecting growth, play their part; second, the position of the ear, the position of the seed, the size of the seed, and other physiological and morphological factors on which development depends cannot be overlooked; and third, the genetic composition of the plant on which the seed matures, differing as it may from the genetic composition of the seed itself, is of great importance. We cannot differentiate and measure all these influences, but the data already presented are sufficient to show that they exist. When one considers the fact that selection must be founded on the average phenotypic differences shown by fraternities of seeds, and that these phenotypic differences are brought about by such complex conditions, he is constrained to admit that theories as to the effect of selection cannot be based on such material.

There is a possibility, however, of showing some of the effects of particular conditions, and this we shall attempt to do.

TABLE 10

A comparison between the protein content of self-pollinated (S.) and of open-pollinated (O.P.) ears.

Pedigree		1912	1913	1914	1915	1916	1917	1918	Average	
14-30-4-3-7-11-18	S.	12.03	15.23	14.77	15.64	15.08	13.29	15.35	14.89	S.
	O. P.	—	13.46	14.78	14.44	13.11	13.47	15.33	14.10	O. P.
14-30-4-4-2-7	S.	12.03	15.23	14.77	16.13	14.26	14.59		14.99	S.
	O. P.	—	13.46	14.78	13.65	12.17	13.74		13.19	O. P.
14-30-6-11-3-11	S.	12.03	15.23	15.87	16.50	16.90	15.93		16.30	S.
	O. P.	—	13.46	11.79	15.59	15.29	15.27		14.49	O. P.
14-30-6-2-13-5	S.	12.03	15.23	15.87	16.22	14.88	—		15.55	S.
	O. P.	—	13.46	11.79	15.44	12.76	14.20		14.10	O. P.
14-30-6-4-3-13	S.	12.03	15.23	15.87	16.96	15.80	—		16.38	S.
	O. P.	—	13.46	11.79	15.63	10.56	14.10		13.10	O. P.
14-30-9-8-1-6	S.	12.03	15.23	14.51	14.47	15.78	—		14.92	S.
	O. P.	—	13.46	11.60	13.08	11.53	11.12		12.07	O. P.
14-30-12-14-1-10	S.	12.03	15.23	14.26	16.20	14.68	—		15.05	S.
	O. P.	—	13.46	12.13	16.31	12.09	13.81		13.51	O. P.
14-4-6-16-2-12-47	S.	12.03	14.58	14.16	13.53	14.25	14.05	15.27	14.31	S.
	O. P.	—	13.30	12.71	12.42	14.89	14.34	15.00	13.78	O. P.
14-4-6-4-7-8	S.	12.03	14.58	14.16	15.36	14.26	14.14		14.59	S.
	O. P.	—	13.30	12.71	14.65	13.79	14.96		14.47	O. P.
14-4-15	S.	12.03	14.58	13.29					—	S.
	O. P.	—	13.30	—					—	O. P.
14-4-20	S.	12.03	14.58	13.50					13.50	S.
	O. P.	—	13.30	11.97					11.97	O. P.
14-4-1	S.	12.03	14.58	14.60					14.60	S.
	O. P.	—	13.30	12.87					12.87	O. P.
14-6-20-10-3	S.	12.03	—	13.01	14.09	15.93	—	—	14.34	S.
	O. P.	—	—	11.61	12.69	15.09	16.75	16.96	13.13	O. P.
14-22-15-1-29	S.	12.03	13.42	15.86	14.47			15.83	14.90	S.
	O. P.	—	13.47	12.60	13.04			14.56	13.42	O. P.
14-8-11	S.	12.03	14.41	13.96					14.19	S.
	O. P.	—	13.20	13.33					13.27	O. P.
20A-8-5-35-66	S.			16.09	16.49	18.01	16.93	18.69	17.32	S.
	O. P.			13.39	14.90	17.30	—	15.27	15.22	O. P.
20A-4-25-47-24	S.			16.09	15.71	16.15	16.06	16.53	16.13	S.
	O. P.	—		13.39	13.90	14.43	—	16.75	15.03	O. P.
20A-11-10-13	S.			16.09	15.39	16.84			16.12	S.
	O. P.			13.39	14.85	17.01			15.93	O. P.
21-13-9-7-57-43	S.		10.24	10.76	9.61	7.67	7.80	7.39	8.91	S.
	O. P.		9.87	7.09	7.20	8.22	7.38	7.30	7.84	O. P.
21-13-2-11-36	S.		10.24	10.76	11.18	10.01	—		10.60	S.
	O. P.		9.87	7.09	8.15	7.36	9.98		7.76	O. P.
30-1-10-8-3	S.			11.64	13.82	14.44	14.40	16.23	14.72	S.
	O. P.			—	12.57	13.15	14.25	15.09	13.77	O. P
30-7-5-10-7	S.			11.64	13.38	13.03	13.06	13.99	13.37	S.
	O. P.			—	11.91	12.28	12.04	13.85	12.52	O. P.
30-15-4-7	S.			11.64	13.29	14.28	13.80		13.79	S.
	O. P.				12.45	13.80	13.83		13.36	O. P.
Average.........	S.		13.58	14.28	14.65	14.57	13.45	14.91	14.24	S.
Average.........	O. P.		12.66	12.16	13.31	13.05	13.25	14.14	13.10	O. P.
Percent decrease of O. P. below S. is 8.01.								Difference	1.14	

Comparison of the protein content of self-pollinated and of wind-pollinated ears

As we shall have occasion to speak both of wind-pollinated ears—called open-pollinated ears—and of self-pollinated ears, in several connections, let us first compare the difference in proteid content observed under the two systems. Table 10 lists 23 families which have been grown from self-pollinated seed for varying periods of from 3 to 7 years.

The number in the left-hand column is the pedigree number for the last year grown. It follows a system we have long used. The first number, 14–30–4–3–7–11–18. means that plant 18 was the daughter by self-pollination of ear 11 of the generation before, granddaughter of ear 7, and so on. If a cross had been made, it would have been indicated by the multiplication sign.

Each year a number of plants of each strain were self-pollinated. The resulting ears were analyzed, and the average protein content listed as "S." The number of ears obtained varied from one to twenty, but in general from five to ten may be taken as the number used. In addition a composite sample of the wind-pollinated ears of the same strain was analyzed. These figures are designated "O. P."

It will be noted that there is a rather constant difference in protein content in favor of the selfed ears. In only one instance, family 14–4–6–4–7–8– (1917), does the protein content of the open-pollinated ears exceed that of the self-pollinated ears by an amount greater than might be expected to arise from experimental error. In this case the difference in favor of open-pollination is 0.82 percent. Occasionally a rather large advantage—over 4 percent—is held by the self-pollinated ears, but on the average it is 1.14 percent,—the grand average of the self-pollinated ears being 14.24 percent and that of the open-pollinated ears 13.10 percent. In general then one may figure that the protein content of a self-pollinated ear must be reduced by 8 percent of the total amount found to make it comparable with the protein content of an open-pollinated ear.

Just why the open-pollinated seed runs consistently lower than the self-pollinated seed in protein is not wholly clear. The only constant differentials are the use of the paper bag and the application of the pollen all at one time in self-pollination, and one would hardly expect so great an effect from these seemingly inconsequential factors. There are two other differentials of a variable nature, however, which undoubtedly account for a considerable proportion of the difference. In the first place the open-pollinated seeds, though related strains have been planted together, may be expected to show more or less heterosis. And, as we shall show later, heterosis increases the weight of the seed and decreases the percent of protein. This phenomenon accounts for a certain range in the proteid differences and for the correlation between self-pollination and high protein; but it does not answer the whole question, for where heterosis is at a maximum in controlled artificial pollinations, as judged by increase in seed weight, the difference in protein content is only about half of that under discussion. Perhaps the remaining difference is due to a relation between composition and the number of seeds produced. In general, though not invariably, the self-pollinated ears contain a less number of seeds, and HAYES and GARBER (1919) have recently noted that there is a high degree (60 percent) of inverse correlation between number of seeds and protein content. Other things being equal, ears with a large number of seeds have a lower protein content than ears with a small number of seeds.

The immediate effect of pollination on the size and composition of the seed

By taking advantage of the phenomenon of xenia, so-called, a number of interesting facts connected with the composition of maize have been discovered. The procedure has been to select two varieties which differ in endosperm color, and in which the endosperm color of the F_1 seed is not the same as that of either parent. It is obvious that a mixture of approximately equal quantities of pollen may be made from two such plants, A and B, and applied to either plant. Distinguishable selfed and crossed seeds will be obtained on the same ear, maturing under a constant environment.

The effect of crossing on weight of seed in sixteen such mixtures is shown in table 11. Strain A is Illinois Low Protein, a white dent; strain B is Stadtmueller's Leaming, a yellow dent selected for high protein through six generations. Sixteen pairs of plants were selected. The respective mixtures were made and applied to each plant from which the mixture of pollen came. On the resulting ears the selfed and the crossed seeds could be separated easily and weighed. The result was that in each of the 32 comparisons the crossed seeds weighed more than the selfed seeds. The average of A × B over A was 3.7 ± 0.23 cg; and the average of B × A over B, 5.9 ± 0.37 cg. There was a percentage increase of 15.3 in the first case, and 24.2 in the second case.

The difference in actual elaboration of material is even greater than these figures show, for in 25 of the 32 cases listed the water content of the crossed seeds is lower than the selfed seeds (see table 12). The differences are not striking, it is true, but they are large enough to be significant. The average decrease in water content of A × B from A is 0.57 ± 0.09 percent; and in the case of B × A and B, is 0.25 ± 0.08 percent.

The cause of the smaller amount of moisture in the air-dried crossed seeds, is not clear. It has been our experience that it is impossible to dry out immature seeds as well as seeds that have matured normally on the plant. But the fact is that the larger seeds resulting from crosses often appear to mature more slowly than selfed seeds borne on the same ear. Consequently the conclusion that the crossed seeds owe their lack of water to a more rapid and complete maturity is admissible only as a possibility. We are inclined to attribute the matter merely to a difference in the physical constitution of the tissues formed, particularly those of the endosperm. If the pericarp is somewhat more porous and the cell walls within the endosperm somewhat thinner in the crossed seeds, rapidity of drying certainly would be facilitated.

TABLE 11

The immediate effect of pollination upon the weight of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Parent plant A	Weight of seeds in centigrams				Parent plant B
		A	A × B	B × A	B	
1	21-13-9-7-57-1	27.0	32.1	30.3	22.3	14-30-4-3-7-11-4
2	-2	20.3	21.9	25.2	21.4	-3
3	-3	26.0	31.1	30.9	22.5	-10
4	-5	22.2	24.3	31.4	25.3	-2
5	-7	26.9	31.1	35.2	28.3	14-30-4-4-2-7-6
6	-10	27.8	32.4	29.9	23.7	14-30-4-3-7-11-1
7	-14	28.0	30.3	39.4	29.5	14-30-4-4-2-7-3
8	-20	30.9	35.5	21.6	21.1	14-30-6-11-3-11-3
9	-24	28.5	33.0	29.1	25.5	14-4-6-4-7-8-5
10	-25	24.6	29.7	36.6	30.1	14-4-6-16-2-12-8
11	-29	32.4	38.4	24.1	19.3	14-30-4-3-7-11-7
12	-31	14.7	17.3	24.3	20.5	-8
13	-33	16.5	18.9	23.6	18.5	-9
14	-35	19.2	23.6	31.3	25.5	-18
15	-36	22.3	25.1	36.4	28.9	14-30-4-4-2-7-14
16	-43	20.6	22.7	34.5	27.3	-2
Average		24.2	28.0	30.2	24.4	
Increase			3.7±0.23	5.9±0.37		
Percent increase			15.3	24.2		

The immediate effect of pollination upon protein content (table 13) is slight. Only 7 instances out of 32 show an increase of protein in the crossed seeds, and there is on the average a decrease that is probably significant; but the decrease is small. The average of A × B is 0.14 ± 0.04 percent less than the average of A; and the average of B × A is 0.60 ± 0.07 percent less than B. When one remembers that the crossed seeds are larger than the selfed seeds, and that the increase in size is greater in B × A than in A × B, it is clear that there is actually more protein produced in the crossed seeds than in the selfed seeds (see table 14). The result of crossing, therefore, is not merely to increase the size of the seed, while the total amount of protein remains the same. The evidence, as far as one may judge from the protein content, is that the increase consists of tissue having practically a normal constitution. At the same time it must be admitted that the increase due to crossing is not uniform in the various parts of the seed, and therefore the increase of one type of protein may be greater than another. This matter is illustrated by the data set forth in table 14.

TABLE 12

The immediate effect of pollination upon the water content of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Parent plant A	Percent of water in seeds				Parent plant B
		A	A × B	B × A	B	
1	21-13-9-7-57-1	7.20	6.90	7.05	7.70	14-30-4-3-7-11-4
2	-2	7.40	6.90	6.80	7.65	-3
3	-3	7.80	7.70	7.22	7.25	-10
4	-5	7.80	7.33	7.13	7.60	-2
5	-7	7.40	6.90	7.15	7.25	14-30-4-4-2-7-6
6	-10	7.18	6.85	6.70	7.55	14-30-4-3-7-11-1
7	-14	8.27	7.55	7.60	7.10	14-30-4-4-2-7-3
8	-20	8.23	6.78	6.65	7.40	14-30-6-11-3-11-3
9	-24	7.45	6.58	6.95	6.95	14-4-6-4-7-8-5
10	-25	7.30	6.83	7.10	6.45	14-4-6-16-2-12-8
11	-29	7.45	7.65	7.32	7.35	14-30-4-3-7-11-7
12	-31	7.30	7.37	7.05	7.20	-8
13	-33	7.53	7.65	7.45	7.30	-9
14	-35	8.45	7.33	7.00	7.55	-18
15	-36	8.48	7.20	7.15	7.25	14-30-4-4-2-7-14
16	-43	8.40	7.05	6.50	7.20	-2
Average		7.73	7.16	7.05	7.30	
Decrease			0.57±0.09	0.25±0.08		

Five pollen mixtures of the kind illustrated in tables 11, 12 and 13, though not the same ones, were used in obtaining A × B, A, B × A and B seeds in which the weights of pericarp, endosperm, and embryo were determined (table 15). The seeds were soaked in boiling water for about 10 minutes, the parts were separated, and dried to a comparatively constant weight in a steam-heated room by exposure to the air in open sacks for one month. The weights of the 10 to 50 seeds comprising each lot were then averaged. The results serve to explain some of the differences obtained in the reciprocal crosses in protein content.

TABLE 13

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Low-protein parent plant A	Percent of protein in seeds				High-protein parent plant B
		A	A × B	B × A	B	
1	21-13-9-7-57-1	7.54	7.25	12.64	13.27	14-30-4-3-7-11-4
2	-2	7.16	7.25	11.80	13.06	-3
3	-3	6.98	6.71	12.33	12.81	-10
4	-5	7.46	7.55	12.59	13.19	-2
5	-7	7.49	7.58	14.95	15.90	14-30-4-4-2-7-6
6	-10	7.21	6.71	13.13	13.73	14-30-4-3-7-11-1
7	-14	7.23	7.10	13.59	14.53	14-30-4-4-2-7-3
8	-20	8.59	8.45	16.00	15.93	14-30-6-11-3-11-3
9	-24	8.51	8.23	14.30	14.51	14-4-6-4-7-8-5
10	-25	7.01	7.31	13.72	13.63	14-4-6-16-2-12-8
11	-29	10.47	9.95	11.60	12.08	14-30-4-3-7-11-7
12	-31	7.35	7.02	11.77	12.12	-8
13	-33	7.30	7.45	12.03	12.88	-9
14	-35	6.89	6.61	13.58	14.74	-18
15	-36	7.17	6.94	14.00	14.22	14-30-4-4-2-7-14
16	-43	6.89	6.99	14.11	15.23	-2
Average		7.58	7.44	13.26	13.86	
Decrease			0.14±0.04	0.60±0.07		
Percent decrease			1.85	4.33		

The average weight of the A × B embryos over the A embryos was only 6.14 percent, as compared with the increase of 28.20 percent by which the B × A embryos exceeded the B embryos. In other words the large embryos characteristic of the high-protein strain were increased only 6.14 percent when crossed with the pollen of the low-protein strain, although the small embryos of the low-protein strain were increased by 28.20 percent. Yet this is somewhat of a distortion of the results due to the difference in size of the embryos in the parent strains. As a matter of fact there was no significant difference between the size of the embryos in reciprocal crosses though perhaps this result might have been expected because of a possible difference in the metabolic efficiency of the plants of the different strains upon which the seeds matured. But A × B gave embryos averaging 3.11 cg in weight, and B × A yielded embryos averaging 3.00 cg in weight,—as nearly an even result as could be expected.

In the case of the endosperms, where two maternal nuclei unite with one paternal nucleus, the result was different. The large endosperm of the low-protein type, 27.75 cg, was increased to 32.41 cg by the cross with A; but the endosperm of the high-protein type, 21.55 cg, was increased to 27.27 cg by B pollen. Thus, though the increase of B × A over B was 4.66 cg or 16.79 percent, the increase of A × B over A was 6.02 cg or 27.93 percent. The reciprocals were not alike, as in the case of the embryos. The B × A endosperms exceeded the A × B endosperms by 5.14 cg. Nevertheless, it should be noted that the single nucleus coming from the low-protein (B) increased the endosperm of the seeds borne on A by 6.02 cg, while the single nucleus of the high-protein (A) increased

TABLE 14

The immediate effect of pollination upon the amount of protein in maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Pollen mixture number	Parent plant A	Amount protein in centigrams				Parent plant B
		A	A × B	B × A	B	
1	21-13-9-7-57-1	2.04	2.33	3.83	2.96	14-30-4-3-7-11-14
2	-2	1.45	1.59	2.97	2.80	-3
3	-3	1.82	2.09	3.81	2.88	-10
4	-5	1.66	1.84	3.95	3.34	-2
5	-7	2.01	2.36	5.26	4.50	14-30-4-4-2-7-6
6	-10	2.00	2.17	3.93	3.25	14-30-4-3-7-11-1
7	-14	2.02	2.15	5.35	4.29	14-30-4-4-2-7-3
8	-20	2.65	3.00	3.46	3.36	14-30-6-11-3-11-3
9	-24	2.42	2.72	4.16	3.70	14-4-6-4-7-8-5
10	-25	1.72	2.17	5.02	4.10	14-4-6-16-2-12-8
11	-29	3.39	3.82	2.80	2.33	14-4-30-4-3-7-11-7
12	-31	1.08	1.21	2.86	2.48	-8
13	-33	1.20	1.41	2.84	2.38	-9
14	-35	1.32	1.56	4.25	3.76	-18
15	-36	1.60	1.74	5.10	4.11	14-30-4-4-2-7 -14
16	-43	1.42	1.59	4.87	4.16	-2
Average		1.86	2.11	4.03	3.40	
Percent increase			13.1	18.5		

TABLE 15

The immediate effect of pollination upon the weight of different parts of the maize seed as shown by selfed and reciprocally crossed seeds grown upon the same ears. Plants grown 1917.

Parent plant A	Parts of seed	Weight in centigrams				Parent plant B
		A	A × B	B × A	B	
14-30-4-3-7-11-5	Embryo	2.68	2.57	2.63	2.10	21-13-9-7-57-6
	Pericarp	2.21	2.57	1.92	2.07	
	Endosperm	21.74	26.79	30.35	24.85	
	Per. and end.	23.95	29.36	32.27	26.92	
	Total	26.63	31.94	34.91	29.02	
14-30-6-2-13-5-1	Embryo	3.04	3.10	2.29	1.82	21-13-9-7-57-9
	Pericarp	—	—	—	1.96	
	Endosperm	—	—	—	22.11	
	Per. and end.	26.71	29.93	29.31	24.08	
	Total	29.75	33.04	31.60	25.91	
14-30-6-2-13-5-11	Embryo	3.30	3.37	3.10	2.26	21-13-9-7-57-15
	Pericarp	2.95	2.99	1.89	1.71	
	Endosperm	22.46	25.32	30.19	25.96	
	Per. and end.	25.41	28.31	32.08	27.68	
	Total	28.71	31.69	35.19	29.94	
14-30-12-14-1-10-4	Embryo	3.05	3.18	2.15	1.92	21-13-9-7-57-22
	Pericarp	1.92	2.15	2.17	1.89	
	Endosperm	21.46	29.08	22.01	22.15	
	Per. and end.	23.39	31.23	24.18	24.04	
	Total	26.44	34.41	26.34	25.96	
14-30-4-4-2-7-7	Embryo	2.62	3.33	4.83	3.64	21-13-9-7-57-39
	Pericarp	2.55	2.93	2.37	2.27	
	Endosperm	20.55	27.91	47.12	43.68	
	Per. and end.	23.10	30.85	49.50	45.95	
	Total	25.72	34.18	54.33	49.60	
Average embryo		2.93	3.11	3.00	2.34	
Increase		0.18 =	6.14%	0.66 =	28.20%	
Average pericarp		2.40	2.66	2.08	1.98	
Increase		0.26 =	10.83%	0.18 =	5.05%	
Average endosperm		21.55	27.27	32.41	27.75	
Increase		6.02 =	27.93%	4.66 =	16.79%	
Average pericarp and endosperm		24.51	29.93	33.46	29.73	
Increase		5.42 =	22.11%	3.73 =	12.54%	
Average total		27.44	33.05	36.47	32.08	
Increase		5.61 =	20.40%	4.39 =	13.68%	

the endosperm of the seeds borne on B by only 4.66 cg. There is a discrepancy between the effects of the two pollens for which we have no adequate explanation, though some light is thrown on the matter by the following computations. If one assumes that the heterotic effect would be the same in both crosses and that disregarding this effect the potentiality of each nucleus entering into the formation of the endosperm is realized, then the resulting endosperm of A × B should be one-third of the sum of 21.55 cg plus 21.55 cg plus 27.75 cg, or 23.62 cg, and the resulting endosperm of B × A should be one-third of 27.75 cg plus 27.75 cg plus 21.55 cg, or 25.68 cg. The endosperm actually produced in the cross A × B was 3.65 cg, or 15.5 percent, above the calculated endosperm, and the endosperm actually produced in the cross B × A was 6.73 cg, or 26.2 percent, above the calculated endosperm. The effect of heterosis was presumably greater, therefore, in the case where the low-protein type was the mother.

The increase in the pericarp may be explained as a simple stretching with resulting increase of tissue formation, due to the larger endosperm which had to be covered. Where the increase in endosperm was large, the increase in the pericarp was large; where the increase in endosperm was small, the increase in pericarp was small.

Another rather interesting experiment was made with some of the same pollen mixtures used in obtaining the results set forth in tables 11, 12 and 13. These combinations of pollen from high-protein (variety 14) and from low-protein plants (variety 21) were applied to the silks of a third variety of high-protein plants (20A). One cannot compute the amount of change in seed size, moisture content or protein content when compared with selfed seeds of parent plants C (20A), but a comparison can be made between the effects of the pollen of the low-protein type, 21, and the high-protein type, 14, on the high protein type 20A. Since strain 20A and strain 21 originally came from the same commercial variety of white dent corn, and strain 14 is a yellow dent, it cannot be assumed that the total amount of genetic difference between 20A and 21 is greater or less than the total amount differentiating 20A and 14. For this reason there is no *a priori* justification in predicting a greater or smaller heterotic effect of either pollen on the third strain. The experiments actually did yield a slightly larger seed when the pollen from strain 14 was used. The difference was 1.4 ± 0.38 cg (table 16). If this may be taken to be the result of heterosis, it is a rather important fact, for table 17 shows that there is no significant difference between the moisture content of the two crosses, yet there is a slight but real excess of protein in favor of strain 14,—a difference of 0.26 ± 0.05 percent.

Whether one is justified in generalizing from so few data is problematical. At least it is permissible to point out a possible inference. Illinois

High Protein (20A) and Illinois Low Protein (21), during the process of selection to change the composition, have been differentiated physically in a very marked degree. They must differ by a great many hereditary factors. Stadtmueller's High-Protein Leaming, except for the yellow color of the seeds, resembles Illinois High Protein much more than the latter resembles Illinois Low Protein. Yet the indication of the heterosis test is that Illinois High Protein is genetically more distinct from Stadtmueller's High-Protein Leaming than it is from Illinois Low Protein in certain factors essential to optimum development of the seed. Thus, while it is undoubtedly true that selection for high protein in varieties of different origin does bring about a certain phenotypic uniformity and possibly a considerable genotypic uniformity, nevertheless different combinations of genes contributing toward high-protein complexes may be isolated, and through their union a notable heterotic effect obtained without reduction in protein content other than that due to increased size and number of seeds.

TABLE 16

The immediate effect of pollination upon the weight of maize seed as shown by out-crossed seeds resulting from application of some of the same pollen mixtures used in tables 11, 12 and 13 to a third high-protein variety. Plants grown in 1917.

Pollen mixture number	Parent plant C	Weight of seeds in centigrams		Parent plant A	Parent plant B
		C × A	C × B		
1	20A-8-5-35-8	20.5	24.5	21-13-9-7-57-1	14-30-4-3-7-11-4
2	-3	19.7	23.7	-2	-3
3	-4	25.4	25.0	-3	-10
6	-11	20.3	22.9	-10	-1
8	-24	27.3	27.5	-20	14-30-6-11-3-11-3
9	-26	25.9	27.7	-24	14-4-6-4-7-8-5
13	-6	20.2	20.1	-33	14-30-4-3-7-11-9
16	-13	20.1	25.8	-43	14-30-4-4-2-7-2
16	-15	23.9	27.5	-43	-2
16	-18	21.6	20.9	-43	-2
16	-21	20.2	18.9	-43	-2
16	-30	21.7	21.2	-43	-2
16	-37	21.6	21.0	-43	-2
17	-7	21.3	22.8	-9	14-30-6-2-13-5-1
Average		22.1	23.5		
Difference			1.4±0.38		

Some further information of a character similar to that just discussed, is given in table 18. Mixtures of pollen from pairs of plants designated

A and B were applied to the plants designated A. The comparison was made by self-pollinating plants B. The table shows the calculations of the percent increase in weight of the crossed seeds over the selfed seeds, and the percent of protein in samples of the selfed and the crossed seeds.

The percent increase in weight of seed in crosses of high protein with high protein vary a great deal, all the way from − 7.30 to 29.23 percent. In at least two of the examples, the small amount of increase is due to variable time of pollination, since small crossed seeds were found only at the tip of some of the ears. But there is also evidence of a real difference in the ratio of increase. For example, the amount of increase when plants of family 14 were pollinated by pollen from plants of family 20A is much greater than when the reciprocal cross was tried. This result is in harmony with the figures obtained when reciprocal crosses were made between families 21 and 14, where the increase was usually greater when the plants of family 14 were used as maternal parents. The explanation would appear to lie in the fact that families 20A and 21 were both originated from Burr's White by selection for high protein and for

TABLE 17

The immediate effect of pollination upon weight, water content and percent of protein in maize seed as shown by out-crossed seed resulting from some of the same pollen mixtures as used in tables 11, 12 and 13. High-protein plants pollinated by a mixture of a distinct high-protein and a low-protein strain.

Pollen mixture number	Parent plant C	Weight of seeds in centigrams		Percent water in seeds		Percent protein in seeds	
		C × A	C × B	C × A	C × B	C × A	C × B
1	20A-8-5-35-8	20.5	24.5	7.25	7.16	16.98	17.30
2	-3	19.7	23.7	7.40	7.25	16.61	16.98
3	-4	25.4	25.0	7.48	7.28	16.35	16.52
6	-11	20.3	22.9	7.10	7.20	17.16	17.65
8	-24	27.3	27.5	7.67	7.63	15.70	15.70
9	-26	25.9	27.7	7.50	7.05	16.36	16.98
13	-6	20.2	20.1	7.30	7.18	13.75	13.74
16	-13	20.1	25.8	6.85	7.00	16.58	17.00
16	-15	23.9	27.5	7.00	6.95	16.33	17.07
16	-18	21.6	20.9	6.98	6.98	12.90	12.77
16	-21	20.2	18.9	7.10	7.00	11.98	12.24
16	-30	21.7	21.2	7.20	7.10	13.34	13.33
16	-37	21.6	21.0	7.40	6.95	13.37	13.37
17	-7	21.3	22.8	7.05	7.44	17.69	18.03
Average..............		22.1	23.5	7.23	7.16	15.36	15.63
Difference.............			1.4±0.38	0.07±0.04			+0.26±0.05

low protein respectively. They seem to have retained the power to affect the plants of family 14 somewhat similarly in spite of their present differences in physical appearance.

TABLE 18

Immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed seed grown upon the same ears (A and A × B seeds from same ears). Plants grown 1916.

	Parent plant A	Percent protein in seeds			Parent plant B	Percent increase in weight of crossed seeds above selfed
		A	A × B	B		
High by low	14-30-9-8-1-3	14.84	14.63	10.63	21-13-2-11-18	16.94
High by high	14-4-6-4-7-26	13.78	13.82	16.22	20A-4-25-37	19.44
	14-4-6-16-7-27	13.14	12.59	16.63	-45	29.23
	-30	12.99	13.04	16.63	-45	8.90
	-28	13.92	13.94	16.63	-45	25.00
	20A-4-25-41	15.95	16.08	14.10	14-4-6-4-7-23	15.29
	-27	16.10	16.53	15.30	-8	−7.30*
	-31	15.61	15.56	16.49	14-30-9-8-1-4	17.48
	-37	16.22	16.27	12.90	14-4-6-4-7-7	6.97
	-45	16.63	16.41	14.44	-24	12.69
	-33	16.69	16.69	16.49	14-30-9-8-1-4	9.91
	-22	16.51	17.20	16.27	-6	−6.86*
	20A-11-10-22	15.65	15.59	—		6.99
	20A-4-25-32	15.85	16.10	16.49	14-30-9-8-1-4	5.36
Average..................		15.31	15.37	15.72		11.01
Low by high	21-13-2-11-23	10.28	10.25	12.90	14-4-6-4-7-7	15.32
	-36	7.91	7.79	14.44	-24	12.57
	-26 (1)	10.51	10.28	14.44	-24	15.33
	-24	8.23	8.36	13.06	-13	14.54
	-26 (2)	11.85	11.98	14.44	-24	13.65
	21-13-9-7-22	7.54	7.39	15.85	14-30-9-8-1-8	16.03
Average..................		9.39	9.34	14.19		14.57

* Crossed seeds at tip of ear.

The increase in size of seed when low-protein plants (family 21) are pollinated with pollen from high-protein plants (family 14) is rather uniform.

The difference in protein content between the crossed and the selfed seeds is not marked. In general it follows the amount contained by the maternal parent.

The protein content of different ears borne on the same plant

In a few instances it has been possible to obtain two selfed ears of similar size on the same plant. Numerous pollinations of this kind were made, but in most cases only one ear developed. As seen by referring to table 19, the difference in protein content is very small with the exception of one plant. Sometimes the upper ear is slightly higher than the lower ear, sometimes the reverse is true. The higher protein content probably follows the ear with the smaller number of seeds. At any rate, position on the plant is not a notable factor in influencing the protein.

TABLE 19

Difference in protein content of ears produced on the same plant through self-pollination. Plants grown 1917.

Plant number	Percent protein in seeds			
	Top ear	Bottom ear	Difference	
21–13–9–7–57–54	7.24	6.97[*]	–0.27	
–58	7.74	8.09	+0.35	
–73	8.72	8.67	–0.05	
–100	11.06	10.91	–0.15	
21–13–2–11–36	7.84	7.91	+0.07	
–26	11.85	10.51	–1.34	
Average..............	9.08	8.84	–0.24	2.64% decrease

[*] The analysis of this ear was given as 13.79 percent and was not checked as it should have been. This is a difference of 6.53 percent on the same plant. This is nearly twice as large as the greatest difference between any two plants grown the same year in this strain, so it seems that the analysis is wrong. The percent of nitrogen was given as 2.02. In another similar wrong-appearing analysis which was checked, the nitrogen was given as 2.30 when in all probability it should have been 1.30. It therefore seems reasonable to suppose that in this case also the figure should be 1.02 instead of 2.02 and has been so calculated.

The immediate effect of pollination on different ears borne on the same plant

Since two ears borne on the same plant appear to have potentially the same protein contents provided the development of each is similar, the experiments reported in tables 20, 21 and 22 are in a sense repetitions of the experiments reported in tables 13 and 17. There is this point of difference, however: The data for tables 20, 21 and 22 were not obtained by mixing pollen of pairs of plants, applying the mixture to the silks of a single ear, and separating the resulting crossed and selfed seeds by inspection, but by applying foreign pollen to the silks of one ear and self pollen to the silks of another ear on the same plant. Conceivably there might be a difference in the results of the two experiments. In the mixed-pollen experiments a large number either of crossed or of selfed seeds developing on a single ear might influence the size or composition of the seeds of the other type. In the development of two ears having different male parents on the same plant, such influence—if any—would be expected to be small. Under such an hypothesis, there should be a greater difference between the seeds A and the seeds A × B in the two-ear experiments than in the mixed-pollen experiments.

TABLE 20

Immediate effect of pollination upon the protein content of the seeds as shown by selfed and crossed ears borne upon the same stalks (A and A × B are different ears on same plant). Plants grown 1916.

High-protein parent plant A	Percent protein in seeds			Low-protein parent plant B
	A	A × B	B	
14–30–9–8–1–2	16.30	12.57	9.91*	21–13–2–11–18
14–6–20–10–7	17.24	13.88	7.28	21–13–9–7–1
14–4–6–4–7–25	14.58	14.43	11.08	21–13–2–11–31
14–4–6–16–7–15	14.00*	15.43	6.97	21–13–9–7–37
7–14	14.00*	12.76	6.97	–37
2–27	14.25*	15.61	7.84	–40
14–30–9–8–1–5	15.78*	16.24	11.69	21–13–2–11–28
14–6–20–10–9	15.93*	17.30	8.79*	21– –y
20A–11–10–16	16.84*	18.28	12.94	10–4–3–4–5–2–1
20A–8–5–43	18.01*	17.55	8.56	21–13–9–7–5
–42	18.01*	17.21	8.56	–5
–17	18.01*	17.27	7.67*	–10
–37	18.01*	16.97	7.67*	–10
–41	18.01*	16.94	7.67*	–23
Average.............	16.36	15.89	8.83	
Low-protein parent plant A				High-protein parent plant B
21–13–2–11–5	10.23	9.44	17.58	20A–8–5–10
–38	12.32	12.81	18.44	–22
Average.............	11.28	11.13	18.01	

* No selfed ear obtained, average of all selfed ears of the same strain used instead.

It does not seem to us that such a conclusion is justified by the facts, though there are a few instances where the difference in protein content between the selfed and the crossed seeds is notably large. The difficulty in the matter is the paucity of evidence. On the plants of table 20, attempts at self-pollination were successful in only 5 out of 16 cases, owing to a variety of circumstances. No selfed ears being obtained from the plants on which the crosses were, a comparison was made between the percent of protein in the cross-pollinated ears and that of the average of all selfed ears of the same strain. This comparison gave results comparable to those obtained with the mixed pollen. The protein content followed that of the maternal parent, though it lagged somewhat behind. Nevertheless, one could not maintain that a selfed ear obtained on the particular plant used would have had exactly the same value as the average of all the self-pollinated sister ears. Hence a strict comparison is invalid.

TABLE 21

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed seed produced on different ears on the same stalk. Plants grown 1915.

Low-protein parent plant A	Percent protein in seeds				High-protein parent plant B
	A	A × B	B × A	B	
21–13–2–2	10.52	11.23	15.29	15.92	20A–4–6
2–12	12.19	12.33	15.37	—	–2
2–6	9.24	8.51	—	—	–1
9–10	10.51	8.59	17.50	—	–9
2–5	12.77	12.68	—	16.12	14–30–6–11–9
2–8	12.35	12.13	—	16.24	–10
2–3	11.08	11.87	—	—	2–6
9–4	10.14	11.02	—	—	11–5
9–3	10.40	11.55	—	—	2–1
9–7	7.81	7.47	—	16.86	–3
9–1	10.38	10.35	16.38	—	20A–11–2
Average...........	10.67	10.70			

The five remaining cases comprised three plants where the cross was high protein by low protein, and two cases where it was low protein by high protein. Plant 21–13–2–11–38 crossed with pollen from plant 20A–8–5–28 was the only example of a crossed ear having a higher percent of protein (12.81) than a selfed ear (12.32). In the remaining ears the percent of protein in the crossed ears was less than in the selfed ears, and in two cases the difference is extreme. Plant 14–30–9–8–1–2 selfed had 16.30 percent protein in the ear; but when crossed with pollen from plant 21–13–2–11–18 from a sib averaging 9.91 percent for selfed ears, the crossed seeds contained only 12.57 percent protein. Likewise plant 14–6–20–10–7 yielded a selfed ear containing 17.24 percent protein, but when crossed with pollen from plant 21–13–9–7–1 (7.28 percent protein selfed), the F_1 seeds contained only 13.88 percent protein.

These differences in favor of selfed ears were so much greater than in any of the similarly produced ears, that it is difficult to accept them as correct. On the other hand such differences may be obtained at times, partly for the reasons outlined previously and partly because of the possiblity that certain seed factors may have an immediate influence in particular crosses which is very different from their effect in others.

The theory of factor complexes having different effects seems the more reasonable in view of the results tabled in table 21. There comparable figures are listed for 11 experiments. That is, the figures for the protein of a plant of group A, in this case low protein, were always obtained from a selfed ear of that group, and the figures for the cross of the general formula A × B were always obtained from a second ear borne on the same stalk. The difference in protein content between the crossed ears and the selfed ears is on the whole negligible. Furthermore, considering the data for each pair of ears separately, the crossed ears show an excess in 5 instances and the selfed ears an excess in 6 instances.

TABLE 22

The immediate effect of pollination upon the protein content of maize seed as shown by two ears borne on the same plant one crossed by high the other by low protein. Plants grown 1916.

High-protein parent plant A	Percent protein in seeds				Low-protein parent plant B	High-protein parent plant C
	A×B	A×C	B	C		
20A-4-25-46	18.14	17.77	11.48	15.30	10-3-7-5-4-2-1	14-4-6-4-7-8
20A-4-25-18	16.98	17.17	9.55	16.27	21-13-2-11-20	14-30-9-8-1-6
Average.........	17.56	17.47	10.57	15.79		

In two experiments we were able to obtain a pair of ears on a stalk, the one crossed by high-protein pollen, the other by low-protein pollen (table 22). In the first instance the ear produced by the crossing with the high-protein pollen was slightly higher in protein than the one crossed with low-protein pollen; in the other case the reverse phenomenon occurred.

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed ears grown upon different plants of the same strain

In connection with the work done to estimate the immediate effect of various matings on the size and protein content of the seed, where environmental factors were largely eliminated, it is interesting to examine the results listed in table 23. Here, it is true, environmental factors have full play as far as this is possible on uniform plots where the precautions usually taken in comparative field test are observed. Nevertheless averages of selfed ears and of crossed ears yielded figures of just about the comparative values that would have been expected from the previous work.

The first three reciprocal crosses reported were between various selections of Illinois Low Protein (21) and Stadtmueller's High-Protein Leaming (14). The fourth cross was between Stadtmueller's High-Protein Leaming and Illinois High Protein (20). Each ear, 168 in all, was hand-pollinated.

TABLE 23

The immediate effect of pollination upon the protein content of maize seed as shown by selfed and crossed ears grown upon different plants. Last four pure strains grown 1912, remainder 1913.

Pedigree number	Condition of seeds	Number of ears analyzed	Percent protein in populations	
			Range	Average
(21–3)	Selfed	12	8.94–11.24	10.24
(21–3 × (14–10)–30)	Crossed	4	9.06–10.83	9.72
(14–10)–30 × (21–3)	Crossed	12	12.31–16.71	14.69
(14–10)–30	Selfed	10	13.14–16.22	15.23
(21–3)	Selfed	12	8.94–11.24	10.24
(21–3) × (14–11)–8	Crossed	4	9.55–11.05	10.12
(14–11)–8 × 21–3	Crossed	7	13.35–15.15	14.21
(14–11)–8	Selfed	11	12.78–15.92	14.41
(21–2)	Selfed	15	7.72–12.57	9.41
(21–2) × (14–10)	Crossed	6	8.76–13.37	11.42
(14–10) × (21–2)	Crossed	10	8.74–14.37	11.66
(14–10)	Selfed	14	8.21–15.94	12.19
(14–11)	Selfed	13	8.52–17.86	11.85
(14–11) × (20–2)	Crossed	10	7.73–13.28	10.92
(20–2) × (14–11)	Crossed	10	10.36–16.89	15.10
(20–2)	Selfed	19	11.95–17.10	14.87

In the first two crosses the average protein content of the crossed ears was somewhat lower than the average of the ears of the maternal parent in every case. In the last two crosses the maternal parents were higher than the crosses in two cases. Our conclusions should really be based on the first two crosses, however, for the analysis of the pure strains of the last two crosses was made from ears grown the previous year.

It was thought unnecessary to report the analysis of each individual ear since in no case were more than 15 ears tested, making standard deviations rather untrustworthy. The averages and the protein range of the ears analyzed are sufficient to show that the protein content of the crosses followed that of the mother, and to indicate that the variability of the ears bearing the F_1 seeds was no greater than in the selfed strains.

The protein content of first-hybrid-generation plants bearing second-hybrid-generation seeds

When one compares the protein content of the seeds borne on plants of the first hybrid generation with that of the pure strains from which they came, naturally there is no chance to eliminate variations due to environment except by growing them under as uniform conditions as possible. There is the further difficulty of comparing the actual populations whose plants furnish the gametes for the cross with the hybrid plants themselves. One of three courses may be pursued. Samples of the true parental populations may be held over a year with resultant loss of vitality in the seeds. Parental populations of one year may be compared with hybrid populations of the next year. Or, self-pollinated daughters of the actual parental populations may be tested at the same time as the hybrid populations. The last course of procedure holds some practical advantages, and is probably not any more inaccurate than the other two because of the uniformity of the inbred parental strains.

The first two tests of this kind were made in 1912, one a cross between two high proteins, the other a low protein by a high protein.

Strain 20–2, Illinois High Protein, ranged from 11.95 percent to 17.10 percent protein in the 19 selfed ears analyzed, with an average of 14.87 percent. It yielded at the rate of 39.7 bushels per acre. This strain was crossed with No. 14, of which two selections grown in 1912 yielded at the rate of 50.1 bushels per acre. The first, 14 (1911 seed), ranged from 8.52 percent to 17.86 percent in the 13 ears analyzed,—an average of 11.85 percent. The second selection, 14 (1910 seed), ranged from 8.21 percent to 15.94 percent (14 ears),—an average of 12.19 percent. The F_1 plants, 20–2 × 14–11, yielded at the rate of 55.1 bushels per acre, and ranged from 9.25 percent to 15.02 percent (12 ears),—an average of 11.85 percent. The parental average in protein was thus 13.45 percent, while the average of the F_1 plants was 1.60 percent lower.

Illinois Low Protein (21–2), ranging from 7.70 percent to 12.57 percent in protein (average 9.41 percent for 16 ears), and yielding 42.3 bushels per acre, was also crossed with strain 14. The result was an F_1 generation yielding 53.3 bushels per acre, with ears ranging from 6.24 percent to 13.03 percent protein (24 ears). The average of the F_1 generation, 9.18 percent, was therefore 1.54 percent below the parental average.

In 1915 some further comparisons between F_1 generations and parental strains were made based upon analysis of hand-pollinated selfed ears. Cross 14–6 × 21–13 was the union of Stadtmueller's High-Protein Leaming (14.09 percent average) and Illinois Low Protein (10.40 percent average). The ears of the F_1 plants averaged only 9.49 percent protein but the cross was particularly vigorous, and yielded at the rate of 112 bushels per acre. The remaining F_1-generation plants tested this year were all high-protein matings. The results are recorded in table 24. The protein ranges are not given, as they are similar to other homologous cultures reported in the paper. One need only note that with high-protein strains crossed together, the protein in the seeds of the F_1 ears is below that of the parents. The yields of the F_1 plants are so much greater than those of the inbred strains, however, that the protein per acre is much larger.

TABLE 24

Effect of crossing upon protein content as shown by the ears produced by first-generation-hybrid plants from crosses between selected protein strains. Analyses made with self-pollinated ears. Plants grown 1915.

Parent strains		Percent protein; average selfed ears					Yield, bushels per acre
♀	♂	1915 ♀	1915 ♂	1914 ♀	1914 ♂	1915 F_1	F_1
20A–1	14–6	15.86	14.09	16.09	13.01	13.71	90
20A–2	14–6	15.86	14.09	16.09	13.01	14.01	93
14–30–4	14–6	15.89	14.09	14.77	13.01	14.66	46
14–30–12	14–8–11	16.20	14.49	14.26	13.96	14.02	47
14–6	14–30–12	14.09	16.20	13.01	14.26	13.81	80
14–6	20A–1	14.09	15.86	13.01	16.09	13.94	72
Average		15.33	14.80	14.54	13.89		71
Average of parents		15.07		14.22		14.03	
Difference between F_1 and average of parents		From 1914 average, −0.19 = 1.33 percent decrease From 1915 average, −1.04 = 6.90 percent decrease					

TABLE 25

Effect of crossing upon protein content and yield as shown by the ears produced by first-generation-hybrid plants from crosses between selected protein strains. Analyses made with open-pollinated ears. Plants grown 1917.

Pedigree numbers		Yield bushels per acre			Percent protein in seeds O. P.		
♀ parent	♂ parent	♀	♂	F_1	♀	♂	F_1
High × high							
20A–4–25–36	14–4–6–4–7	34.6	23.2	108.6	14.21	14.96	13.02
20A–4–25–1	14–30–6–4–3	63.7	25.7	112.6	11.51	14.10	10.66
14–4–6–16–7–28	20A–4–25–45	59.2	67.0	107.9	13.43	14.72	13.10
14–4–6–4–7–26	20A–4–25–37	23.2	55.1	110.8	14.96	13.48	12.36
14–4–6–16–7–27	20A–4–25–45	45.5	67.0	124.2	12.95	14.72	12.98
Average.......		45.2	47.6	112.8	13.41	14.40	12.42
Average of parents......................................					13.91		
Decrease of F_1 below average of parents 10.71 percent.........							−1.49
High × low							
20A–4–6	21–13–2–2	—	—	118.2	—	—	8.30
20A–4–25–18	21–13–2–11	55.1	42.1	105.9	13.48	7.07	9.05
14–30–9–8–1	21–13–2–11	49.1	42.1	127.5	11.12	7.07	8.43
14–30–9–8–1	21–13–2–11	49.1	42.1	(156.4)*	11.12	7.07	8.81
14–6–20–10–7	21–13–9–7–1	20.3	65.2	97.2	16.75	6.63	9.36
Average.......		43.4	47.9	112.2	13.12	6.96	8.79
Average of parents......................................					10.04		
Decrease below average of parents 12.45 percent.............							−1.25
Low × high							
21–13–9–7–5	14–4–6–16–2–7	56.3	45.3	101.6	6.70	11.91	8.41
21–13–9–7–18	14–4–6–16–2–12	71.7	45.5	103.6	6.41	13.99	9.25
21–13–9–7–10	14–4–6–16–2–12	76.6	45.5	122.9	6.21	13.99	8.45
21–13–9–7–7	14–4–6–16–2–12	72.8	45.5	120.2	6.46	13.99	8.09
21–13–2–11–5	20A–8–5–10	55.8	56.3	120.0	7.07	12.93	10.49
Average.......		66.6	47.6	113.7	6.57	13.36	8.94
Average of parents......................................					9.97		
Decrease below average of parents 10.33 percent.............							−1.03

* Calculated from imperfect stand with few plants, probably too high, not included in average.

As we have seen, the protein content of seed matured under bags after hand-pollination is approximately 8 percent higher than that of ears of the same strains after wind-pollination, although the immediate effect of the pollen is negligible. This factor should be taken into consideration when the remaining comparisons between F_1 and parent strains are studied, for these later analyses were all made upon open-pollinated seed.

Fifteen tests were made in 1917,—five each of high protein by high protein, high protein by low protein, and low protein by high protein. The data obtained are in many ways preferable to the comparisons made between hand-pollinated ears. A larger number of ears were used, reducing the error of random sampling; the ears were of a more uniform size; the seeds were more numerous; and the maturation of the seeds was on the whole better. The results, shown in table 25, are simply a corroboration of those obtained earlier.

TABLE 26

Protein content of two high-protein types and of first, second and third seed generations of crosses between them. Analyses made with self-pollinated ears.

Pedigree number	Seed generation	Year grown	Number analyzed	Range of protein percentage	Average of protein percentage
14	P_1	1912	13	8.21–18.95	12.02
(20–2)	P_1	1912	18	11.95–17.10	14.87
(20–2) × 14	F_1	1912	12	9.25–15.02	11.85
[(20–2) × 14]–3	F_2	1913	21	12.39–15.89	14.22
[(20–2) × 14]–8	F_2	1913	16	13.21–16.10	14.83
[(20–2) × 14]–3–14Y	F_3	1914	6	14.70–16.63	15.84
[(20–2) × 14]–3–14W	F_3	1914	5	15.05–16.77	15.96
[(20–2) × 14]–3–15Y	F_3	1914	5	14.75–16.10	15.31
[(20–2) × 14]–3–15W	F_3	1914	6	14.53–16.81	15.33

The similarity of the tests within each quintet, makes it necessary to discuss the averages only. We may note first that the F_1 ears are always lower in protein than the average of the parental strains, and that this decrease is rather uniform. In actual percent protein, it is highest in the "high × high" crosses and lowest in the "low × high" crosses, yet the difference is only 0.46 percent. When the difference is reckoned on the mean percent protein, the situation changes. The "high × low" crosses show a decrease of 12.45 percent of the percent of protein carried by the parents, with the other two classes showing 10.71 percent and 10.33 percent respectively. Averaging the results, gives an expectancy of a decrease of a little over 11 percent in protein below the average of the parents in any cross between inbred types. There is, as PEARL and BARTLETT (1911) and HAYES (1914) maintained, a semblance of a dominance of low protein, but the matter is not so easy to interpret. The percent of protein in the F_1 ears is about the same whether the low protein is the maternal or the paternal parent, and it stands nearer to the low-protein than to the high-protein parent; but when one considers the crosses between high-protein strains, it is evident that this decrease can be interpreted as an effect of heterosis. Consider the yields of the high-protein strains and their hybrids. The extreme vigor of the hybrids causes a yield of more than double the "pure" types. Thus in spite of the lower protein content, the total amount of protein per acre in the hybrids is twice as large as in the parent strains. If then one increases the percent of protein in the "low × high" and "high × low" crosses by 11 percent of the amount found to correct for heterosis, the percent protein in the hybrid would be somewhat closer to that of the high-protein parent (see also table 26).

Conclusions regarding the inheritance of protein in maize

There is some advantage in pausing at this point to bring together the odds and ends of data regarding inheritance of protein in maize, before discussing the remainder of the experiments.

In the first place it is perfectly clear that the external conditions, the factors of environment, have such a marked effect on the protein content of maize that it may be raised or lowered as much as 40 percent above or below the total percent produced under average growing conditions. This conclusion may be drawn from a study of change in direction of the protein curve in the high-protein and the low-protein strains grown by the ILLINOIS AGRICULTURAL EXPERIMENT STATION, from the fluctuations from year to year in our own selected strains, from the difference in protein content between hand-pollinated and wind-pollinated ears belonging to the same strain, and from the protein content of two self-pollinated ears from the same stalk.

No doubt the protein content of maize is affected by each and every environmental factor which has an influence on the development of either the plant as a whole or the seed in particular. For example, some lack of nitrogen might appear to affect the development of the stalk and leaves more than the seeds, and some lack of phosphorus might appear to affect the seeds more than the remainder of the plant, but it seems likely that each plays its part in protein synthesis. These various factors cannot have their influences separated and their individual effects described at present, and it probably would not make matters a great deal clearer if this could be done in the rough manner which would necessarily be inherent in such analysis. The factors of environment work together as do the parts of a machine, and though a greater or smaller degree of efficiency in one part of the machine has its effect, the absence of that part stops the machine. There is one thing that may be emphasized, however. A departure from the optimum temperature and moisture at critical periods of the plant's growth appears to overshadow other features in influencing the constitution of the grain. When some of the other conditions are not at their best a plant produces smaller ears or a less number of seeds without there being any great interference with the normal development of the chemical constituents, but let there come a radical diminution in the available moisture or an extreme temperature change after these organs have been laid down normally, and the effect on development is very great. Nevertheless even under such handicaps, it would seem that nearly the normal amount of protein is developed. The percent of protein is influenced, of course, but it is influenced largely through the diminished elaboration of starch.

Taking these facts as we find them, one can realize what great errors may be made in selection. Seeds due to contain 12 percent protein under a hypothetical "normal" environment, may contain anywhere from 9 percent to 15 percent protein because of the conditions under which they develop; mass selection of desirable phenotypes is therefore of less value than with any other character with which we have had experience.

Admitting that proteid variations in maize are to a great extent due to the modifications imposed by a fluctuating environment, one need only study the work of the ILLINOIS AGRICULTURAL EXPERIMENT STATION to realize what a rôle heredity plays in the matter. Then comes the important question: Can one estimate the number of differentiating hereditary factors involved and describe the method by which they are inherited? If a precise answer to this question is desired, it must be no. But the situation is not as discouraging as this answer indicates. Some definite conclusions can be drawn which are of real practical value.

The number of hereditary factors affecting protein elaboration by which varieties of maize may differ must be large. This is an indefinite statement, it is true; but what is meant is that the facts will hardly admit the presumption that they may one day be analyzed by the assumption of five or six differentiating determiners. Possibly the main factors involved are some such small number, but apparently there are modifiers that may run into the hundreds.

The evidence in the case is indirect; at the same time, it is valid. In the first place there are the data of OSBORNE showing the complexity of the protein situation. There are several kinds of proteids. The proteids are very different in their chemical nature. And they are distributed throughout the various tissues which go to make up the seed. Second, the work of the ILLINOIS STATION as well as our own investigations show noteworthy physical differences accompanying change in protein content. Numerous different size relations may be obtained between embryo and endosperm, and between the various tissues making up the endosperm. Size of seed may be thirty times as great in one case as in another. Ear size, number of seeds, shape of seeds, etc., each plays its rôle. Third, the ILLINOIS STATION, starting with a single variety previously brought to a considerable degree of uniformity through selection for physical characters, has been nearly twenty-five years isolating their high-protein and low-protein types without coming to the point where there seems to be no hope of further differentiation.

These facts all point to an involved hereditary complex, a large number of multiple factors affecting protein in the species as a whole. On the other hand, the inexact method of work used at the ILLINOIS STATION, makes it unwise to multiply unduly in our imagination the heterozygous factors involved in their material. Such mass-selection experiments might be carried on for many generations without reaching the end desired when only four or five hereditary factors were under consideration. In fact, the great changes in protein content obtained after only two or three generations of selection in our own experiments because of the control of both parents, lead us to believe in a relatively small number of "main" factors. But the number of subsidiary factors,—factors playing minor rôles,—is by no means small.

The mathematical possibilities involved in recombinations of multiple factors (see EMERSON and EAST 1913) is now a matter of common knowledge. Moreover theory has been corroborated by practical results. Tests have been made on scores of animals and plants and the results reported in numerous scientific papers during the past decade. If such a simple scheme of interpretation could be used for the inheritance of protein, at least an outline of the method of transmission could be made without difficulty. But we are confronted with a much more complicated matter than the cases previously described, due to the protein content of the seed being in part in the embryo and in part in the endosperm, as has already been noted. This is a difficulty inherent in breeding all the cereals, yet it is a difficulty that has been overlooked except for passing mention in one or two papers of the senior author.

The basis of all hereditary transmission, in all such breeding work, is of course the zygote which comes into being with the fusion of two gametes, ♀ + ♂; but at the same time that the zygote is formed the endosperm is laid down by three gametes carrying the same qualities, ♀ ♀ ♂. By successive cell divisions the seed is formed. Now 20 percent of the protein of the maize seed is contained in the ♀ ♂ embryo through which all transfer of hereditary qualities is made, while 80 percent of the protein is found in the ♀ ♀ ♂ endosperm which can have no part whatever in hereditary transmission. One can simplify matters to some degree, however, if he keeps in mind that the size of the embryo and the percent of protein it contains were raised but slightly in the early experiments of the ILLINOIS STATION. The notable variations appeared in the endosperm. The problem, therefore, is the mechanism by which a ♀ ♂ embryo transmits characters which are exhibited in a ♀ ♀ ♂ endosperm. Transmission through the zygote presumably is by the usual methods. Gamete formation is typical. Segregation and recombination occur as in other species, and gametes combine to form zygotes by chance.

It would seem as if no argument need be made in favor of the assumption that the seed is the unit and not the ear. The seed is the new organism, and all of our modern biological evidence leads us to suppose that the seed is formed as described above, and that *many different* hereditary possibilities may be contained in the seeds of a single plant. On the other hand this does not preclude the probability that the genetic constitution of the plant on which the seed matures has a marked influence on its size, shape and composition. In a word the phenotype of the seed may be influenced by the mother no matter what is the genetic composition of the individual seed. The uniformity of the seeds of a single ear in shape and size, the comparative lack of variability in composition of the seeds of a single plant lead to this view.

Keeping these fundamental ideas in mind, what conclusions can be drawn from our data?

Only three facts seem to stand out as important. The chemical composition is influenced by heterosis. This influence on the seed is slight but significant, resulting in a somewhat larger size and concurrent decrease in percent of protein. The influence on the seeds borne by hybrid plants is much greater. The plants themselves being more vigorous than those of the parental strains, the seeds they bear are larger and more numerous, and contain a much smaller percent of protein. Second, the influence of the factors borne by a male gamete are practically without immediate influence on the seed they help to form. Third, the protein content of the seeds of an F_1 hybrid, when corrected for the influence of heterosis, is intermediate between that of the two parents with a tendency to be somewhat closer to that of the high-protein parent.

These facts force us to one of two conclusions. Either the prompt reaction of the two maternal nuclei utilized in the inception of the endosperm has a controlling influence on chemical composition; or, the genetic constitution of the mother plant is the major determining factor. We cannot deny an influence to the immediate reactions within the $3x$ endosperm cells of any seed due to their own individual genetic constitution. There is a demonstrable heterosis as an immediate effect of pollination. There is production of pigments,—at least one ether soluble and at least two water soluble. There is change in the physical character of the starch (horny or floury). Therefore the individual genetic constitution of a seed must effect real changes from the very beginning of the life history. One can hardly call these changes radical, however, when compared with those caused by the genetic constitution of the mother plant. There is no adequate reason for supposing the effect of the two maternal nuclei is more than double the effect of the paternal nucleus, and as far as the change in composition is concerned the latter is almost negligible.

By way of parenthesis it may be said here that this conclusion appears to have considerable theoretical importance. BATESON'S work on the inheritance of pollen color and shape, and the work of EAST on pollen color and self-sterility in Nicotiana have shown the genetic constitution of the mother to be the effective agent. The hereditary factors carried by the gametes seem to have no function during the period of gametic generation. They are passive. The activities of the gametes, their size, shape and color, are determined by the mother's complex.

EAST assumed that the individual constitutions of the gametes were negligible during the haploid generation, that their inheritance was held in abeyance until the formation of the zygote, then to come to the fore to play a rôle in the ontogeny of the organisms.

The data cited in this paper, however, appear to point to a delayed use of individual powers, so to speak, even after the zygote is formed. The individuality of the organism seems to gain momentum as the life history progresses. The genetic constitution by which a seed may differ somewhat from its mother, the inherited individuality which it has received, does not become apparent in the early stages of life. The mother still controls during the time the seed is being matured, parasitically as it were, on the body of its parent, and presumably during the early stages of independent life while stored nutriment is still being utilized.

Returning to the subject in hand, let us summarize our conclusions. Besides the practical difficulties arising from the influence of varying factors of environment, maize breeding for seed characters, and the breeding of other cereals as well, is complicated by a most exaggerated lack of correlation between individuality and performance, between phenotype and genotype. One must select by the characters possessed, which are largely influenced by the constitution of the mother plant, yet the characters which the adult plant will possess are determined by the union of nuclei *both* of which may differ widely in potentialities from those possessed by the plant on which it grew.

An example will perhaps make this clear. It is wholly theoretical and diagrammatic. Let us suppose that the differential factors between two plants, a high-protein and a low-protein plant, let us say, are represented by independent factors *A*, *B*, *C* and *D*. The high-protein plant is *AA BB CC DD*; the low-protein plant is *aa bb cc dd*. A cross is made reciprocally. Except for a slight decrease in protein content due to heterosis the composition of the F_1 seeds follow the mother plant. Seeds *Aa Bb Cc Dd* from the high-protein mother, are high in protein; seeds of the same genetic constitution, *Aa Bb Cc Dd*, from the low-protein mother, are low in protein. In a general selection experiment (starting with unknown pedigrees) one would undoubtedly breed from the former; one could obtain the same end results by breeding from the latter.

Samples from either of these F_1 populations are grown. The average protein content of the ears produced is about the same,—lower than the average of the pure strains entering into combination,—because of heterosis. The seeds vary individually in their protein content, but most of this variation is due to size, position on ear, etc. Only a small proportion of the variation is due to the genetic constitution of the individual seeds themselves. Aside from variations due to the extraneous causes mentioned, the ears are fairly uniform. The protein content has followed the mother. Yet by ordinary recombination the productive capacity is manifold. There are eighty-one ($= 3^4$) actual classes, counting both homozygotes and heterozygotes. And the same troubles ensue in later generations, though in a somewhat lesser degree.

This illustration gives food for thought in connection with cereal breeding. One realizes just why the work carried on by the Experiment Stations in cereal breeding has been so comparatively unproductive.

Presumably scientific methods have yielded no better results than earlier empirical methods. The reason is not far to seek. The later methods have been just as blind, just as empirical as the former. It is also clear why the workers at the ILLINOIS STATION misinterpreted the effects of selection. Selection was endowed with a creative power because of the length of time close selection without pollen control could be carried on without eliminating hope of further progress, and because dispersion indices were not reduced when determined on population averages of seeds (ears). This study, we hope, has done something toward clarifying matters by pointing out the source of the difficulties. But this is not all. There is a method of breeding which may be followed by which results can be obtained in a much shorter time Some of the indefiniteness and blindness of the Illinois method can be eliminated. By its use we have obtained some rather remarkable increases in protein content in a few generations. Furthermore we were able to keep up, and even to increase the yields of the standard varieties used. In the work at the ILLINOIS STATION, the yields were so greatly reduced through inbreeding that they were unprofitable. Their yields for the last six available years are shown in table 27.

TABLE 27

Yields in the chemical-selection experiments of the Illinois Agricultural Experiment Station, 1913–1918. Bushels per acre.

Year	High protein	Low protein	High oil	Low oil	Control variety
1913	30.2	35.5	31.0	23.9	39.6
1914	36.2	43.3	37.4	48.7	55.2
1915	42.4	57.2	45.2	49.9	53.5
1916	14.6	29.6	16.7	19.8	28.2
1917	48.9	56.3	55.9	51.3	63.9
1918	38.8	47.8	46.6	58.2	62.8

We do not maintain that it is desirable to undertake breeding for high protein, or other chemical constituents, as a practical method of increasing food value or industrial utility, but it can be done in the following manner. Self-pollinate large numbers of plants artificially. Test the seeds produced by each individual as accurately as possible by the progeny-plat method. With the continuation of inbreeding if a large enough series be tested, near-homozygous plants having the ability to produce high-protein seeds will be obtained. Some of the crosses between such types will have a high yield and will retain the power to produce large quantities of protein. To be sure the percent of protein in the vigorous hybrids will not equal the percent in the purified parent strains. But relatively the percent will be high, and actually the protein per acre will be rather remarkable.

EXPERIMENTS ON BREEDING FOR HIGH PROTEIN

Original experiments on selection

Selection experiments after the pattern of those conducted at the ILLINOIS AGRICULTURAL EXPERIMENT STATION, were begun in Connecticut in 1906. Seed was selected and a number of ears analyzed from three standard varieties, one a dent, the other two flint. Frequency distributions constructed from these data are shown in table 28. They are given merely to throw a little additional light on the amount of protein as found in unselected varieties grown in Connecticut.

TABLE 28

Frequency distribution of the protein in the ears of certain Connecticut-grown varieties of maize. Analyses on open-pollinated ears.

Variety	Class centers in percent of protein														
	Year	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0
Stadtmueller's Leaming...	1906 seed				10	15	6	4	3	2					
	1907 seed						4	6	8	13	10	5	0	0	2
	1908 seed						1	3	12	13	3	6	1	1	
	1908 crop	2	8	12	18	17	20	21	20	13	4	5	1	1	
Hopson's Longfellow.	1906 seed									1	25	11	5	5	1
	1907 seed							5	9	16	11	6	1		
Sturges's Hybrid......	1906 seed						19	14	26	12	12	7	5	1	

Note: Sturges's Hybrid was produced by crossing a North Carolina Dent with King Philip Flint and selecting toward a twelve-rowed flint type. The corn in 1906 after 8 or 10 years of selection was true to the flint type, and 84 ears out of 96 were twelve-rowed. There were 5 with 10 rows, 6 with 14 rows, and one with 16 rows.

No protein selections were made on the variety known as Sturges's Hybrid, which was a twelve-rowed flint. Hopson's Longfellow, an eight-rowed flint, was selected again in 1907, and Stadtmueller's Leaming was grown for three years. The results, omitting details, are found in the table.

It was soon found, from discoveries made in an extended investigation on heredity in maize, that this method of procedure was hopeless. It had no scientific basis, and carried with it no prospects of the production of a variety which would elaborate a high percentage of protein and at the same time give large yields of grain. This work was discontinued, therefore, until 1912 when the experiments on heredity and on the effects of inbreeding and cross-breeding had proceeded far enough to give some idea of the correct methods to pursue. Two lines of breeding were then started, the one a series of selections in self-fertilized lines, the other a series of selections in alternately crossed and selfed lines. They will be described in order.

Selections in self-fertilized lines

Selections in self-fertilized lines were made on four varieties,—Stadtmueller's Leaming, Burwell's Flint, Illinois High Protein and Illinois Low Protein. No great amount of work was done on any one variety, for the expense of such an investigation is considerable and the available resources were small. Nevertheless the data show conclusively that by breeding successively from self-fertilized ears, strains high in protein can be obtained in a relatively short time.

Stadtmueller's Leaming

Table 29 shows the results obtained between 1912 and 1918 on Stadtmueller's Leaming. The data can be followed easily by referring to the number of the "mother ear" planted. Twenty-seven self-pollinated ears were obtained from planting variety No. 14. These were analyzed. The percent protein varied from 8.21 to 17.86, with an average of 12.03. From the ears highest in protein of this population, five lines were started. The first family in the table descended from ear 14–6. The next selection was ear 14–6–20. The second selection was ear 14–6–20–10. From the population produced by this ear in 1916, two ears were grown. One was ear 14–6–20–10–3, grown in 1917; the other was ear 14–6–20–10–15, grown in 1918.

Passing down the table, the second selection from the population of 1912 was ear 14–30. In 1915, the seeds from two sister ears of 1914 were planted. These are numbered 14–30–4–3 and 14–30–4–4. Thus two lines branch off from ear 14–30–4 in that year, and the ancestors of ear 14–30–4–4 can be followed by referring to the family tabled above.

The major extreme in the population of 1912 was an ear containing 17.86 percent protein. If a larger number of ears had been analyzed, an ear still higher in protein might have been expected. It is clear then that a commercial variety unselected for high protein may contain

TABLE 29

Selection of Stadtmueller's Leaming for high protein in self-fertilized lines.

Subject matter	1912	1913	1914	1915	1916	1917	1918
Mother ear planted	No. 14		14–6	–6–20	–20–10	–10–3	–10–15
Protein in mother ear	—		17.86	16.21	15.92	17.30	16.59
Range S.-P. population	8.21–17.86		9.87–16.21	11.82–15.92	13.47–17.30	—	—
No. S.-P. ears analyzed	27		4	11	10	—	—
Ave. S.-P. population	12.03		13.01	14.09	15.93	—	—
Ave. O.-P. population	—		11.61	12.69	15.09	16.75	17.07
Mother ear planted	Ditto	14–30	–30–4	–4–3	–3–7	–7–11	–11–18
Protein in mother ear		15.80	15.59	14.93	16.18	16.47	14.74
Range S.-P. population		13.14–16.22	13.74–15.52	14.17–16.68	13.39–16.47	12.08–15.05	14.10–16.75
No. S.-P. ears analyzed		10	8	11	10	13	9
Ave. S.-P. population		15.23	14.77	15.64	15.08	13.29	15.35
Ave. O.-P. population		13.46	14.78	14.44	13.11	13.47	15.33
Mother ear planted	Ditto	Ditto	Ditto	–4–4	–4–2	–2–7	
Protein in mother ear				15.52	16.64	15.82	
Range S.-P. population				15.37–16.64	12.40–15.92	12.45–15.90	
No. S.-P. ears analyzed				4	7	8	
Ave. S.-P. population				16.13	14.26	14.59	
Ave. O.-P. population				13.65	12.17	13.74	
Mother ear planted	Ditto	Ditto	–30–6	–6–2	–2–13	–13–5	
Protein in mother ear			16.22	16.03	16.16	15.90	
Range S.-P. population			15.18–16.29	14.49–17.39	13.30–15.90	—	
No. S.-P. ears analyzed			4	6	5	—	
Ave. S.-P. population			15.87	16.22	14.88	—	
Ave. O.-P. population			11.79	15.44	12.76	14.20	
Mother ear planted	Ditto	Ditto	Ditto	–6–4	–4–3	–3–13	
Protein in mother ear				15.96	17.45	16.30	
Range S.-P. population				15.65–17.72	15.04–16.30	—	
No. S.-P. ears analyzed				6	7	—	
Ave. S.-P. population				16.96	15.80	—	
Ave. O.-P. population				15.63	10.56	14.10	
Mother ear planted	Ditto	Ditto	Ditto	–6–11	–11–3	–3–11	
Protein in mother ear				16.29	18.33	16.90	
Range S.-P. population				15.31–18.33	—	—	
No. S.-P. ears analyzed				7	1	1	
Ave. S.-P. population				16.50	16.90	15.93	
Ave. O.-P. population				15.59	15.29	15.27	

TABLE 29 (continued)

Subject matter	1912	1913	1914	1915	1916	1917	1918
Mother ear planted	No. 14	14–30	–30–9	–9–8	–8–1	–1–6	
Protein in mother ear	—	15.80	15.63	15.49	14.59	16.27	
Range S.-P. population	8.21–17.86	13.14–16.22	13.28–15.49	12.35–17.46	14.67–16.49	—	
No. S.-P. ears analyzed	27	10	11	8	7	—	
Ave. S.-P. population	12.03	15.23	14.51	14.47	15.78	—	
Ave. O.-P. population	—	13.46	11.60	13.08	11.53	11.12	
Mother ear planted	Ditto	Ditto	–30–12	–12–14	–14–1	–1–10	
Protein in mother ear			16.09	15.63	14.11	16.77	
Range S.-P. population			12.67–15.63	13.73–18.64	12.23–16.77	—	
No. S.-P. ears analyzed			6	9	10	—	
Ave. S.-P. population			14.26	16.20	14.68	—	
Ave. O.-P. population			12.13	16.31	12.09	13.81	
Mother ear planted	Ditto	–14–22	–22–15			–15–1	–1–29
Protein in mother ear		14.83	15.43			15.46	15.14
Range S.-P. population		11.81–15.43	—			12.42–15.66	14.75–16.39
No. S.-P. ears analyzed		16	2			13	13
Ave. S.-P. population		13.42	15.86			14.47	15.83
Ave. O.-P. population		13.47	12.60			13.04	14.56
Mother ear planted	Ditto	14–8	–8–11				
Protein in mother ear		14.75	15.92				
Range S.-P. population		12.78–15.92	13.16–14.61				
No. S.-P. ears analyzed		11	6				
Ave. S.-P. ears analyzed		14.41	13.96				
Ave. O.-P. population		13.20	13.33				
Mother ear planted	Ditto	14–4	–4–6	–6–4	–4–7	–7–8	
Protein in mother ear		14.73	15.48	14.62	16.50	15.30	
Range S.-P. population		11.90–15.77	13.65–14.68	14.25–16.95	12.90–15.30	13.51–14.68	
No. S.-P. ears analyzed		14	10	11	17	11	
Ave. S.-P. population		14.58	14.16	15.36	14.26	14.14	
Ave. O.-P. population		13.30	12.71	14.65	13.79	14.96	
Mother ear planted	Ditto	Ditto	Ditto	–6–16	–16–2	–2–12	–12–47
Protein in mother ear				14.68	15.62	15.82	14.75
Range S.-P. population				12.20–15.62	11.90–15.82	13.63–14.75	14.71–15.88
No. S.-P. ears analyzed				10	10	10	21
Ave. S.-P. population				14.04	14.25	14.05	15.27
Ave. O.-P. population				12.42	14.89	14.34	15.00

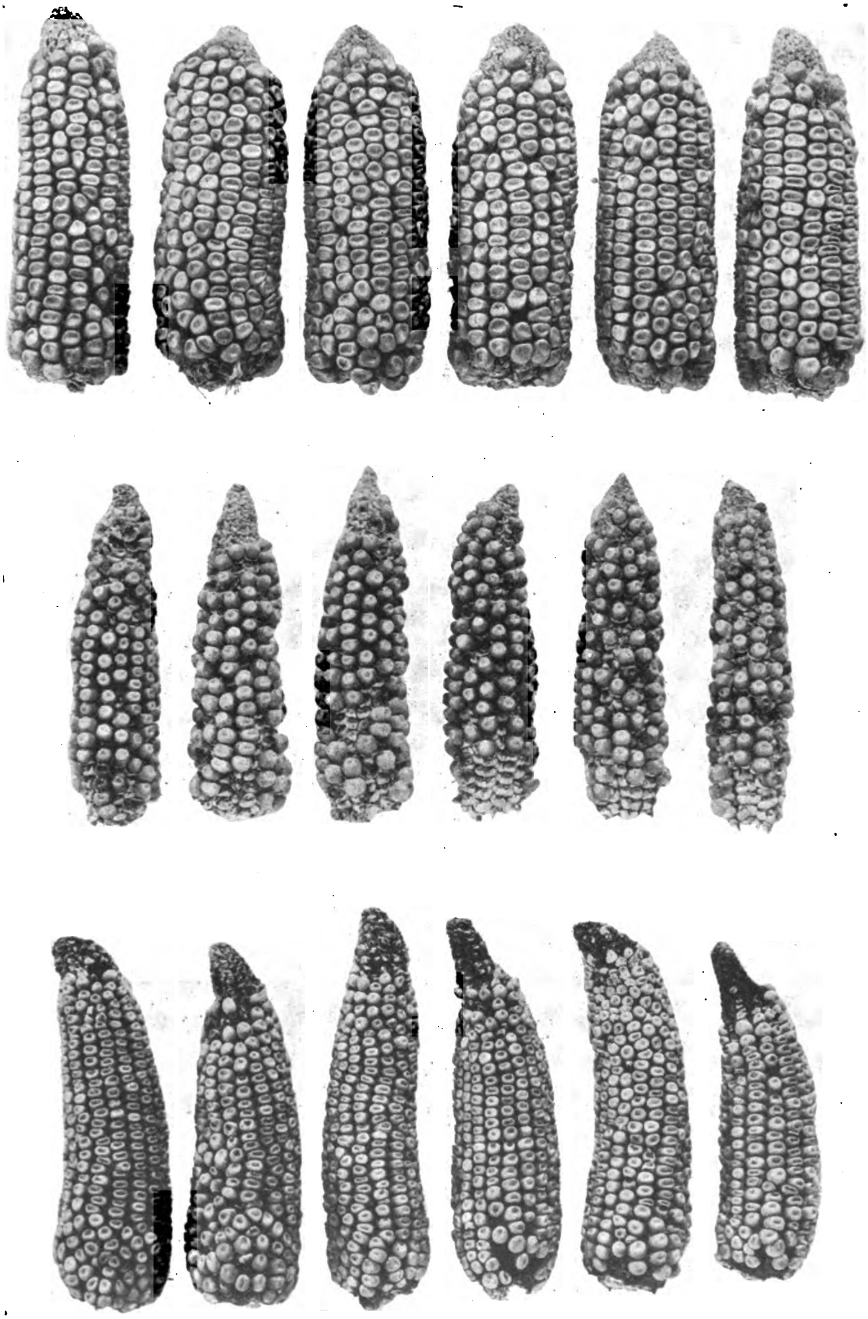

FIGURE 4.—Self-fertilized lines of Stadtmueller's Leaming selected for high protein. Above 14-4; center 14-22; below 14-30.

ears which are very high in protein. And when one considers the fact that these analyses are made on populations of seed in which the parentage of the individual cannot be controlled, there is reason to believe that almost any commercial variety contains hereditary factors which when brought together in a homozygous condition will produce ears as high in protein as those the ILLINOIS STATION has secured after nearly a quarter of a century of mass selection from open-pollinated ears.

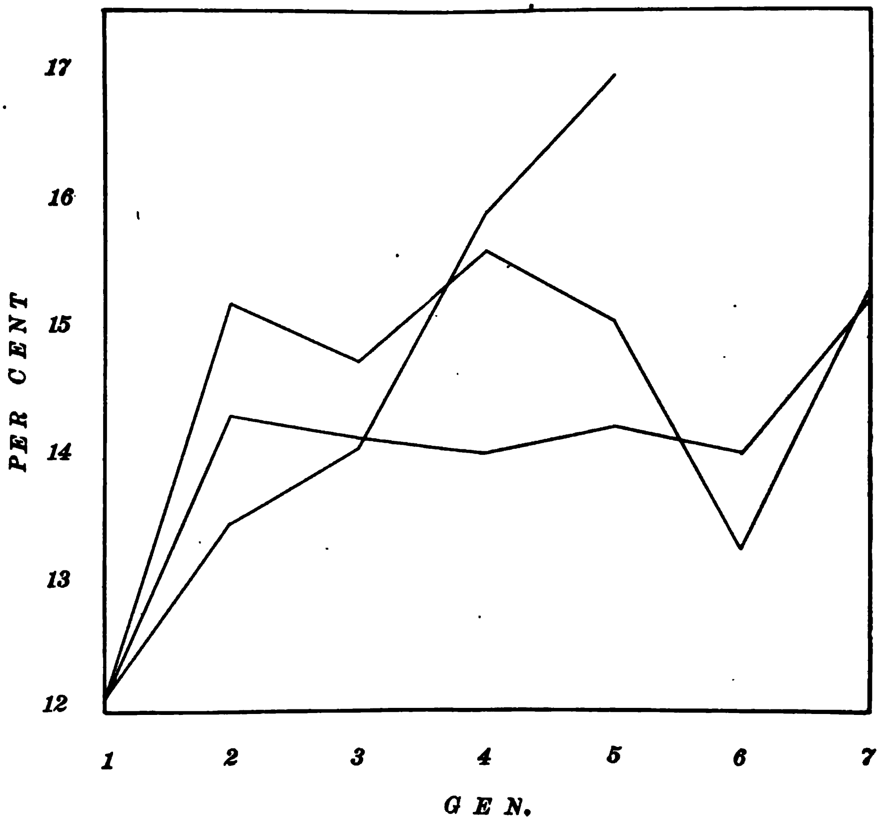

FIGURE 5.—Graphical representation of the results of selecting Stadtmueller's Leaming for high protein in self-fertilized lines.

The highest percentage of protein obtained in any one year's crop at the ILLINOIS STATION was 15.66 in 1916. In 1918 family 14–6–20–10–15 of Stadtmueller's Leaming contained 17.07 percent protein. This amount came as the result of five years of selection. Since the fluctuations from year to year are so wide, one could not rest assured that this strain would continue to produce quite as high a percentage of protein as this; but the fact that it produced 15.09 percent of protein in 1916, and that a sister ear this crop produced 16.75 percent in 1917, makes the quality of performance of this family rather certain.

Perhaps the next best average selection is found in the family ending in ear 14–30–6–11–3–11. The last three years the crops averaged 15.59, 15.29 and 15.27 percent respectively. Some other families did almost as well, but to these two must be given the prizes for protein production. Reference must be made to table 29, if one really wishes to make a comparative study of the strains. Further description in the text is superfluous if this is done; if not, any description is likely to be inadequate.

Illinois High Protein

Selections from the Illinois High-Protein strain were grown at various times between 1906 and 1914 from seed kindly sent to us by Professor L. H. Smith, but not until 1914 were regular analyses made of the ears produced. In that year a mixture of a small quantity of seed from each of a number of ears from the Illinois High-Protein crop of 1913 was grown. Only a few self-fertilized ears were obtained, but of these nine were analyzed. They averaged 16.09 percent, and ranged from 14.97 percent to 16.64 percent. A mixed sample of the open-pollinated ears contained 13.39 percent, which does not indicate a particular aptness for protein production in the Connecticut conditions.

Three selections were grown, two of which were carried on for five years. Table 30 shows the results.

The selfed strains from this material, (including Illinois Low Protein) were better from a developmental standpoint than any of the others included in the experiments. They also reached a comparatively static condition of uniformity more quickly. This was to have been expected, however, for the Illinois strains having been selected through the mother plants rather closely for sixteen generations must have made some approach toward homozygosity in their various characters. Nevertheless it is interesting to note that the experience of the strain during the years of selection in Illinois had not eliminated all possibilities of improvement. In other words, they still exhibited some heterozygosity. This fact may be shown in three different ways. First, there was a reduction in vigor due to the more intense inbreeding of self-fertilization. Second, the two families 20A–4 and 20A–8 are so different from one another that they can easily be distinguished either by the plants or by the ears. No. 20A–8 is smaller in size of plant, earlier to flower, and produces more abundant pollen. The seeds are more corneous and are scarcely dented at all. Third, the advance in protein content from selection in one of the self-fertilized lines is remarkable. The ratio of selective elimination was very small compared to that of the ILLINOIS STATION, for we did not have the facilities for analyzing large numbers of ears; yet, there is no question but that progress in the isolation of a high-protein strain sped more rapidly after selection by this method was begun. Family 20A–8 was different in this respect from family 20A–4. The curve of the latter family shows little change from year to year. Presumably it was more nearly homozygous in the beginning.

TABLE 30

Selections of Illinois High Protein for high protein in self-fertilized lines.

Subject matter	1914	1915	1916	1917	1918
Mother ear planted	20A	20A–8	–8–5	–5–35	–35–66
Protein in mother ear	—	16.64	18.32	18.56	18.60
Range S.-P. population	14.97–16.64	15.32–18.32	17.52–18.97	13.83–18.60	16.35–20.49
No. S.-P. ears analyzed	9	10	11	10	23
Ave. S.-P. population	16.09	16.49	18.01	16.93	18.69
Ave. O.-P. population	13.39	14.90	17.30	—	15.27
Mother ear planted	Ditto	20A–11	–11–10		
Protein in mother ear		16.34	16.57		
Range S.-P. population		13.94–16.57	15.51–18.56		
No. S.-P. ears analyzed		12	11		
Ave. S.-P. population		15.39	16.84		
Ave. O.-P. population		14.85	17.01		
Mother ear planted	Ditto	20A–4	–4–25	–25–47	–47–24
Protein in mother ear		16.27	16.23	16.91	16.31
Range S.-P. population		14.90–16.23	14.93–16.91	15.36–16.63	15.60–17.91
No. S.-P. ears analyzed		10	12	4	22
Ave. S.-P. population		15.71	16.15	16.06	16.53
Ave. O.-P. population		13.90	14.43	—	16.75

Illinois Low Protein

The same argument may be made in the case of Illinois Low Protein. The two families raised came from a single open-pollinated ear of 1912. The two lines separate in 1915. Family 21–13–2–11–36 reaches the year 1917 with a protein content of 9.98 percent, but family 21–13–9–7–57–43 in 1918 had only 7.30 percent protein. The latter family was a rather constant low-protein performer, and if it had been possible to analyze a large number of ears one can hardly doubt a still more rapid drop. The low limit of 6.41 percent obtained in a self-fertilized ear of 1918 is an indication of what might have been expected (see table 31).

It may be worth while to note here that these particular families of Illinois Low Protein were characterized by deficient pollen production. One line was lost because pollen sufficient for producing self-fertilized ears could not be obtained.

TABLE 31

Selection of Illinois Low Protein for low protein in self-fertilized lines.

Subject matter	1913	1914	1915	1916	1917	1918
Mother ear planted....	21	21–13	–13–2	–2–11	–11–36	
Protein in mother ear..	—	9.24	10.16	8.62	7.91	
Range S.-P. population.	8.94–11.24	10.00–11.50	8.62–14.18	7.91–12.32	—	
No. S.-P. ears analyzed.	12	6	11	15	—	
Ave. S.-P. population..	10.24	10.76	11.18	10.01	—	
Ave. O.-P. population..	9.87	7.09	8.15	7.36	9.98	
Mother ear planted....	Ditto	Ditto	–13–9	–9–7	–7–57	–57–43
Protein in mother ear..			10.80	7.81	6.68	6.89
Range S.-P. population.			7.81–12.81	6.68–10.15	6.89–11.06	6.41–9.28
No. S.-P. ears analyzed.			11	16	20	20
Ave. S.-P. population..			9.61	7.67	7.80	7.39
Ave. O.-P. population..			7.20	8.22	7.38	7.30

Burwell's Flint

It has always been the impression among maize-breeders that flint varieties in general average somewhat higher than dent varieties in protein. In fact both the average of all flint varieties and the maximum for flint varieties are somewhat greater than the average and the maximum for dent varieties in JENKINS and WINTON'S (1892) compilation. Moreover our own analyses of Hopson's Longfellow show considerably more protein than those of Stadtmueller's Leaming (table 28). Mindful of this fact selection in self-fertilized lines was undertaken with a standard Connecticut variety known as Burwell's Yellow Flint.

The results (table 32) were not as satisfactory as one might wish. In a series of 19 self-fertilized ears, daughters of self-fertilized ear No. 30, there was only a range of from 7.40 percent to 13.68 percent protein. This was not very promising material. The average of 11.64 percent in self-fertilized ears, probably a percent or two higher than those of the open field, was really lower than that of any flint we have analyzed. But as a means of demonstrating the practicability of the method of breeding, one variety was as good as another, and as this variety was above the average in productiveness, it was used.

Three lines were split off in 1915 and continued for four years. Each of the families yielded to selection but not in the same degree. The banner selection was family 30–1 in which the protein content rose continuously at almost a uniform rate. Based on self-fertilized ears, the average protein content rose from 11.64 percent to 16.23 percent, a gain of 4.59 percent of actual protein content or over 40 percent of the protein originally contained in the variety. Since the range of protein in the 10 selfed ears analyzed in this strain in 1918 was low, and since the maximum was greater than had been found previously in the variety, the prospect of obtaining a really efficient protein producer in Burwell's Flint is thus fairly good.

TABLE 32

Selection of Burwell's Flint for high protein in self-fertilized lines.

Subject matter	1914	1915	1916	1917	1918
Mother ear planted.........	30	30–1	–1–10	–10–8	–8–3
Protein in mother ear.......	—	13.68	14.67	16.51	15.36
Range S.-P. population......	7.40–13.68	12.74–14.67	12.08–16.51	12.96–15.56	15.81–17.00
No. S.-P. ears analyzed......	19	10	9	10	10
Ave. S.-P. population.......	11.64	13.82	14.44	14.40	16.23
Ave. O.-P. population.......	—	12.57	13.15	14.25	15.09
Mother ear planted.........	Ditto	30–7	–7–5	–5–10	–10–7
Protein in mother ear.......		13.48	14.36	14.14	13.78
Range S.-P. population......		11.39–14.36	11.70–14.14	12.24–13.78	13.10–15.31
No. S.-P. ears analyzed......		10	10	10	13
Ave. S.-P. population.......		13.38	13.03	13.06	13.99
Ave. O.-P. population.......		11.91	12.28	12.04	13.85
Mother ear planted.........	Ditto	30–15	–15–4	–4–7	
Protein in mother ear.......		13.24	14.28	15.85	
Range S.-P. population......		12.59–14.28	12.68–15.85	13.17–14.97	
No. S.-P. ears analyzed......		10	10	8	
Ave. S.-P. population.......		13.29	14.28	13.80	
Ave. O.-P. population.......		12.45	13.80	13.83	

Conclusions regarding selection for high protein in self-fertilized lines

MENDEL'S original paper showed that the result of self-fertilization without selection on any allelomorphic pair *Aa* is to reduce the number of heterozygotes so that in the *n*th generation the ratio is $1 : 2^n - 1$. Equal fertility for all plants and random mating of gametes is of course assumed.

EAST and HAYES (1912) generalized this expression, for independent inheritance showing that the probable number of homozygotes and of any particular class of heterozygotes is expressed in the formula

$[1 + (2^r + 1)]^n$, where r is the segregating generation and n is the number of allelomorphic pairs. JENNINGS (1916) stated the same formula somewhat differently.

In 1917 JENNINGS considered the numerical results of breeding when genes are linked, and found that while the formulae are complex, the general result in self-fertilization is to decrease the number of heterozygotes and to increase the number of homozygotes.

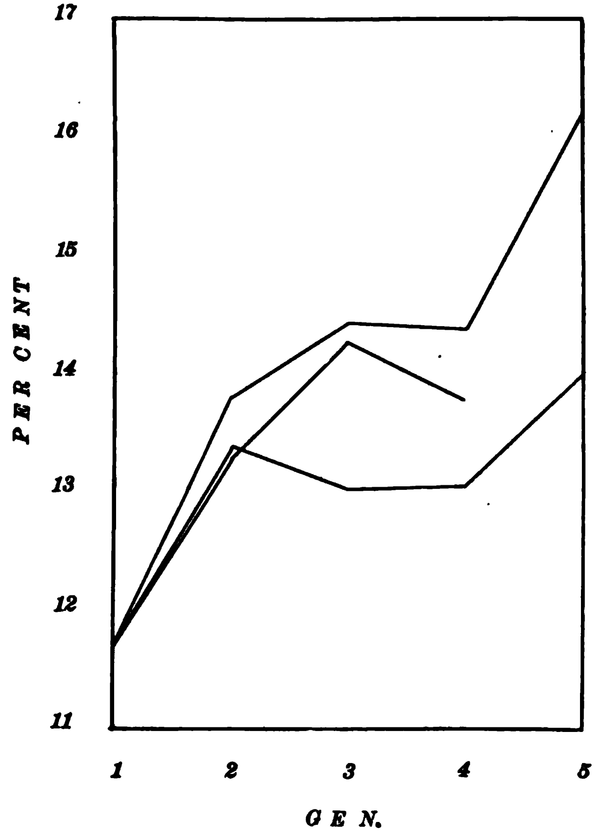

FIGURE 6.—Graphical representation of the results of selecting Burwell's Yellow Flint for high protein in self-fertilized lines.

More recently JONES (1918), by studying the variability of various characters in maize in successive self-fertilized generations, has demonstrated that these mathematical generalizations actually hold in practice.

The data presented here are merely a corroboration of these results on another character complex. There are indeed many complications in the inheritance of protein, as has been emphasized in the preceding pages.

Nevertheless, it can be stated without hesitation that methods of self-fertilization will give high-protein strains of maize in shorter time than any other procedure. We need only call attention, therefore, to one or two practical points in connection with the strains thus produced.

In the first place, there is no direct correlation between protein content and heterosis. In fact, the correlation, if any, is negative. In other words, inbreeding for protein does not have to overcome the obstacles which stand in the way of breeding for yield in that yield is a function of vigor and vigor dependent to some extent on heterosis. On the other hand, inbreeding reduces the vigor, and with it the size of the ear, the size of the seeds and the number of the seeds per ear. Since there is an inverse correlation between size and number of seeds and protein content, the percent of protein actually found in our inbred strains is higher than may be expected in high-yielding strains with the same potential protein production. The same statement holds for such closely bred strains as those produced by the long-continued selection experiments of the ILLINOIS AGRICULTURAL EXPERIMENT STATION, though there the vigor has not been depressed as it would have been by a like number of generations of self-pollination. The facts being as they are, however, we must take them into consideration in any practical method of breeding for high protein, for in order to have any desirability whatever in agricultural practice a strain of maize or any other crop must have a high yield. Without the power of yielding high returns, no commercial variety can survive, be its particular qualities what they may. To try and surmount these difficulties, several experiments were undertaken where the strains under observation were alternately selfed and crossed.

Selected matings between high-protein plants

One of the major difficulties in producing high-protein strains by inbreeding is the lack of a practical method for making rigid selections based upon large numbers. In other words straight selection on a small scale does not begin to exhaust the possibilities inherent in such a variable cross-fertilized plant as maize. Theoretically, one should test out all the extreme individuals in a very large population; but this is impracticable. A partial solution of the problem was found, however, in a plan by which selected high-protein plants were crossed together.

This plan is based upon the plausible assumption that since the various inbred high-protein strains differ in their morphological features, similar protein percentages may be due to different genetic constitutions. The

TABLE 33

Effects of crossing and selection upon protein content 1914. Analyses of cross-pollinated ears between inbred strains of high-protein corn. Plants grown 1914.

Plant number ♀	×	Plant number ♂	Percent protein	Crossed ears planted 1915
14-4-1-6	×	14-30-9-7	14.66	
-10	×	14-6-2	13.23	
-8	×	14-30-12-8	14.67	
-4	×	14-6	13.42	
-3	×	14-6-1	14.09	
-9	×	14-30-4-16	14.07	
-12	×	14-8-11-2	13.75	
-15-12	×	14-8-11-4	13.32	
-5	×	14-6-7	12.89	
30-4-16	×	14-4-1-9	12.10	
-6	×	(20 × 14)-3-14W-1	12.90	
-12	×	14-6-13	15.54*	E
-6-8	×	14-6	13.93	
-9-3	×	14-6-3	13.80	
-7	×	14-4-1-6	11.53	
12-10	×	14-6-17	13.10	
12-8	×	14-4-1-8	12.55	
-16	×	14-8-11-6	15.31*	F
-6	×	14-6	15.02	
14-6-3	×	14-30-9-3	13.78	
-6	×	14-30-12-6	11.89	
-9	×	14-8-11-5	12.99	
-8	×	14-4-1-3	12.77	
-2	×	14-4-1-10	12.31	
-1	×	14-4-1-3	15.38	
-7	×	14-4-15-15	12.59	
-5	×	14-30-6-8	14.83	
-17	×	14-30-12-10	10.71*	G
-15	×	20	13.33	
-16	×	20	9.08*	D
-22	×	(20 × 14)-3-14-12	12.17	
-4	×	(20 × 14)-3-14-1	13.15	
14-8-11-5	×	14-6-9	14.84	
20	×	14-6-15	17.98*	A
	×	14-6-16	18.48*	B
(20 × 14)-3-14-1	×	14	15.27	
-1	×	14-30-4-6	16.37*	C

* Selected for growing 1915.

procedure was simply to cross different selected high-protein lines, to self-pollinate the first-generation plants, and to select again from the progenies which represent segregating generations. Cross matings were made between individual plants of several such second-generation families in as large numbers as possible, using each plant both as a male and as a female. Since it had already been determined that the immediate effect of pollination was small, the analysis of the seed was used to indicate the value of the individual in reciprocal crosses. Thus the crosses selected for continuation by self-fertilization were those in which both parents were high-protein performers.

The results from the first year's work carried on in this manner are set forth in table 33. From among the families of Stadtmueller's Leaming (14) and Illinois High Protein (20) which had been selected for high protein in self-fertilized lines during three or more years, thirty-seven crosses were made. The protein contents of the ears produced ranged from 18.48 percent to 9.08 percent,—the minor extreme possibly being an error of analysis. The results of the analyses are practically the same as they would have been had self-fertilized ears of the mother plants listed been analyzed. Nevertheless, since reciprocal pollinations were made in several cases, it is clear that we were dealing with individuals which had the ability to store up rather large quantities of protein. In 1915, seven of these ears were planted.

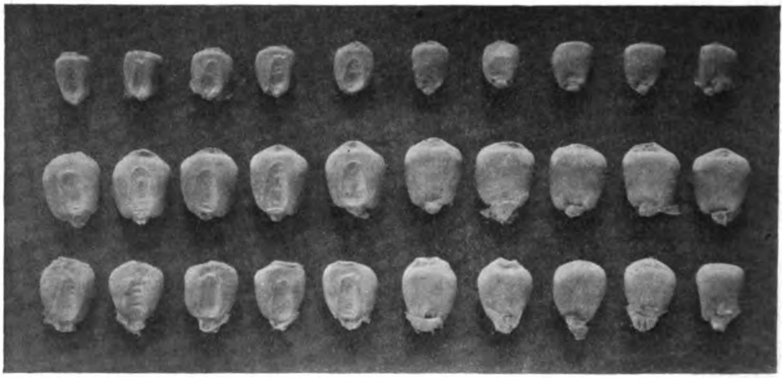

FIGURE 7.—Above, Illinois High Protein; below, Illinois Low Protein; center, F_1 generation.

Table 34 presents the results of analyzing ten selfed ears from the vigorous plants produced by these high-protein extremes. On the whole there is not much choice in the various lots. Lot C with ears ranging from 16.32 percent to 13.99 percent is the best. Its average protein content was 15.33 percent, not a bad average, though 1 percent lower than the parent ear.

TABLE 34

Effects of crossing and selection upon protein content. Analyses of first-generation crosses between high-protein strains. Plants grown in 1915.

Pedigree number	Designation	Range of percent protein in seeds of individual selfed F_1 plants										Average	Percent protein of parent ear
		Highest ear									Lowest ear		
20 × (14-6-15)	A	15.86*	14.81	14.36	14.26	14.22	14.17	13.71	13.45	12.81	12.46	14.01	17.98
20 × (14-6-16)	B	16.50*	14.81	14.69	14.44	13.59	13.29	13.04	13.04	12.07	11.61	13.71	18.48
(20 × 14)-3-14W-1) × (14-30-4-6)	C	16.32*	16.14	15.78	15.52	15.47	15.14	15.04	14.99	14.88	13.99	15.33	16.37
(14-6-16) × (20)	D	16.38*	15.29	15.08	14.69	14.21	14.10	14.02	12.64	12.41	10.57	13.94	9.08
(14-30-4-12) × (14-6-13).	E	15.92*	15.48	15.08	15.17	14.94	14.63	14.49	13.86	13.68	13.39	14.66	15.54
(14-30-12-16) × (14-8-11-6)	F	15.13*	14.89	14.62	14.59	13.95	13.69	13.38	13.36	13.34	13.23	14.02	15.31
(14-6-17) × (14-30-12-10)	G	15.55*	14.57	14.54	14.52	13.96	13.55	13.52	13.28	12.42	12.20	13.81	10.71

* Selected for growing 1916.

TABLE 35

Effect of crossing and selection upon protein content. Analyses of cross-pollinated ears grown on F_2 plants from the highest F_1 ears. Plants grown in 1916.

Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein	Plant number ♀ ♂	Percent protein
A2 × B2	14.16	B1 × C3	16.65	C3 × B1	14.73	D2 × A3	16.55	E2 × D6	14.26	F2 × D6	17.10	G1 × F2	12.50
A4 × D2	14.77	B2 × A4	18.57*	C7 × B7	14.64	D4 × A6	11.44	E4 × D6	13.30	F3 × D6	15.74	G2 × F2	12.10
A5 × C7	14.88	B3 × C7	16.78	C10 × B7	11.07	D6 × A5	9.76	E6 × D6	13.58	F4 × D6	15.49	G3 × F2	14.86
A6 × D2	14.15	B4 × C10	13.90	C11 × B7	10.75	D9 × B10	9.78	E8 × D4	9.28	F5 × A14	10.93	G15 × F15	10.74
A8 × D2	12.13	B6 × C42	16.82	C13 × B20	14.99	D10 × B10	9.43	E16 × C22	14.26	F13 × A8	14.28	G17 × F15	13.58
A10 × D2	15.41*	B7 × A4	16.22	C22 × B20	11.45	D12 × A5	9.34			F15 × A8	12.68	G19 × F15	10.25
A11 × D2	14.74	B9 × A4	17.70	C23 × B20	18.16*	D15 × A4	15.28			F17 × A8	15.05	G21 × F15	14.88
A13 × D4	15.75	B10 × A23	15.75	C27 × B7	13.39	D18 × A11	15.21			F19 × A8	16.46	G31 × F29	10.73
A14 × D18	11.81	B12 × A23	12.83	C28 × B22	12.21	D21 × A11	16.26			F21 × A8	10.74	G39 × F31	12.10
A15 × D4	17.43	B16 × D15	16.38	C42 × B22	12.01	D22 × A11	14.09			F22 × A8	12.66	G40 × F31	15.08
A17 × D18	11.12	B20 × D15	18.35*			D24 × A11	15.15			F23 × A8	15.79		
A18 × D18	13.43	B22 × C42	11.19			D25 × A15	11.67			F27 × C27	13.14		
A19 × D18	14.56	B27 × A25	17.00			D26 × A15	12.61			F29 × O.P.	12.02		
A22 × B2	15.23*	B28 × A25	16.04			D27 × A15	16.35*			F31 × G32	13.23		
A23 × D18	14.66	B38 × C42	12.62			D29 × G19	15.60						
A25 × D18	13.39	B41 × A46	16.15*			D30 × A18	12.79						
A26 × B2	16.21*					D31 × G31	15.92						
A28 × D21	15.81*					D34 × A4	16.72						
A36 × D18	17.85*					D35 × A36	12.13						
A38 × D30	16.69					D39 × A36	14.79						
A41 × D18	16.07					D41 × A4	16.14						
A46 × D30	15.71					D43 × A41	9.72						
A47 × D35	16.46												
A48 × D35	17.35												
Average	14.99	Average	15.81	Average	13.34	Average	13.49	Average	12.94	Average	13.95	Average	12.68
Parent A	15.86	Parent B	16.50	Parent C	16.32	Parent D	16.38	Parent E	15.92	Parent F	15.13	Parent G	15.55

* Selected for growing 1917 based upon the protein content of both parents.

The major extreme of each lot was planted in 1916, and a series of crosses made between the resulting plants (see table 35). The protein content was determined on 101 of these crosses. As mother plants, lot B proved to be the best,—the average for the ten ears tested being 15.81 percent. The second highest average came from lot A used as mothers, —an average of 14.99 percent based on 24 ears. Two ears having over 18 percent protein were obtained, and ears with over 17 percent were numerous. Those ears marked with an asterisk were planted the spring of 1917.

Several ears were selfed from each of these ten selections. The analytical results are shown in table 36. The columns of the table are in descending order of the protein content of the parent ears. If now we note the order of the average protein content of the offspring we find it is thus: 1, 5, 4, 10, 2, 6, 7, 3, 8, 9. In other words the plus half of the parents produced four-fifths of the plus half of the offspring, and the minus half of the parents likewise produced four-fifths of the minus half of the offspring. The parent-offspring correlation is so high that one cannot doubt the great influence of heredity on the result. The general result is that three strains of corn have been produced after seven years' work, one averaging 16.31 percent, the second averaging 15.42 percent, and the third averaging 15.05 percent in protein. They have considerable vigor, give fair yields of grain, and at the same time are as high in protein as most of the straight self-fertilized families. In these strains, moreover, there is a reserve of genetic variability out of which it is possible theoretically to carry the percent of protein to a still higher level.

The two ears highest in protein content from family (C23 × B20) were planted in 1918 (see table 37). From the plants of lot (C23 × B20) − 8, the fifteen selfed ears obtained averaged 17.68 percent protein. The average was thus higher than the parent ear by a slight margin. The majority of the ears were over 18 percent in protein, and one reached the extraordinary figure of 20.14. Here, then, was a fairly high-yielding partially inbred strain which in the single year of 1918 probably averaged over 16 percent in protein in the ordinary field run of ears.

The second lot of plants, daughters of lot (C23 × B20) − 6 was not quite as good as the other, still an average for fifteen selfed ears of 16.39 percent protein would have been considered exceptional had the sister strain not been in existence. Four of the fifteen ears were over 18 percent in protein.

Two other lots of seed were grown as checks. No. 170 was a seed mixture taken from the three highest ears of *each* of the ten selections grown in 1917. The average protein content was 15.84 percent. The second lot, No. 171, was a seed mixture taken from one ear each of the three *highest* of the ten selections of 1917. The average protein content was 16.22. Thus again heredity shows its ruling hand. Selections from the plants having the higher protein contents, produced plants giving the higher protein contents.

Conclusions regarding breeding for high protein

For one acquainted with that vast reservoir of genetic variability—the maize plant—emphasis as to its breeding possibilities has an empty sound. It is sufficient to say that no one knows the limits of progress when breeding to increase or to decrease any one of its characters. What we have to say regarding breeding for high protein, therefore, concerns breeding methods rather than breeding limits.

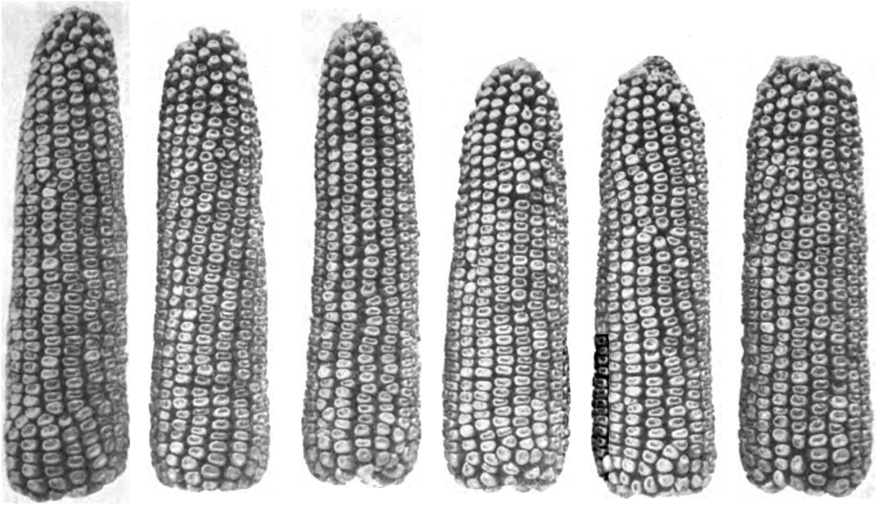

FIGURE 8.—Ears of F_1 hybrid plants (14–30 × 14–6), a cross between two families of Stadtmueller's Leaming selected for high protein. Average protein content, selfed ears, 14.66 percent.

High-protein maize can be secured in the shortest possible time and with a minimum expenditure of effort only when selection is based upon an accurate control of the true biological units and when the germ-plasm contributed by each sex is given due consideration and equal opportunity of expression. In practice the basis of such a method is self-fertilization.

The results obtained from continued selection for high protein in self-fertilized lines depend almost exclusively upon the heredity of the original plants chosen as progenitors. Unless adequate possibilities for recombination are thus present, no amount of selection can create the qualities sought, since there is no evidence of frequent mutation. Obviously the chances for success do not depend wholly upon the number of individuals used, and the rigidity of selection. External conditions must be such as will bring out the highest expression of the desired character, and correlation between personal characteristics and genetic constitution must be fairly high; but given these conditions, progress depends upon the magnitude of the operations at the beginning rather than at the end.

In reality this statement is but a rephrasing of old genetic postulates, and their application to the specific problem of breeding maize for high protein. But what of the result? A high percentage of protein may be produced by this method with certainty and rapidity; yet high percentage composition does not insure high production per unit of area. There is a certain amount of antagonism between high yield and high protein; and even if this were not the case, selection for one character alone would tend to be at the neglect of the other. Merely as a matter of probability it would be more difficult to secure a high proportion of certain ingredients together with high yield than it would be to secure either alone. But the truth is that inbred strains showing the highest percent protein are weak and unproductive. As a rule high-protein strains are less vigorous than strains not so selected, and crosses between them generally give lower yields than other crosses. It may well be, therefore, that high-protein maize can be secured only at the expense of maximum total production. Whether it is worth while to produce special types of maize with increased proportions of certain ingredients in spite of their reduced yields, need not be discussed here.

Such results as are possible can be obtained most easily, we think, by combining strains obtained by self-fertilization and selecting again from the recombinations obtained. Protein content is due to a large number of inherited factors; and various strains having the same percentage composition probably differ in respect to the factors inherited, so that there is a real chance for progress in their union. Since at the same time such a method increases productiveness by means of heterosis it thus serves two purposes.

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Prepared by Dr. J. BEN HILL of the Botany Department, THE PENNSYLVANIA STATE COLLEGE, State College, Pennsylvania, and HELEN DEUSS HILL. Page numbers printed in black-faced type refer to headings of articles or of sections. Numbers in italics indicate references included in lists of "Literature cited."

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